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(54) Title: USE OF ANTAGONIST ANTI-CD40 ANTIBODIES FOR TREATMENT OF AUTOIMMUNE AND INFLAMMATORY DISEASES AND ORGAN TRANSPLANT REJECTION

(57) Abstract: Methods of therapy for treating a subject for an autoimmune disease and/or inflammatory disease are provided. The methods comprise administering a therapeutically effective amount of an antagonist anti-CD40 antibody or antigen-binding fragment thereof to a patient in need thereof. The antagonist anti-CD40 antibody or antigen-binding fragment thereof is free of significant agonist activity, but exhibits antagonist activity when the antibody binds a CD40 antigen on a human CD40-expressing cell. Antagonist activity of the anti-CD40 antibody or antigen-binding fragment thereof beneficially inhibits CD40L-mediated stimulation of CD40-expressing cells, and thus inhibits survival and signaling pathways of human CD40-expressing cells that are mediated by CD40L.



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USE OF ANTAGONIST ANTI-CD40 ANTIBODIES FOR TREATMENT OF  
AUTOIMMUNE AND INFLAMMATORY DISEASES AND ORGAN  
TRANSPLANT REJECTION

FIELD OF THE INVENTION

The invention relates to methods for treatment of autoimmune and inflammatory diseases using antagonist anti-CD40 monoclonal antibodies.

5

BACKGROUND OF THE INVENTION

CD40 is a 55 kDa cell-surface antigen present on the surface of normal and neoplastic human B cells, dendritic cells, other antigen presenting cells (APCs), endothelial cells, monocytic cells, CD8<sup>+</sup> T cells, and epithelial cells. The CD40 antigen  
10 is also expressed on activated T cells, activated platelets, inflamed vascular smooth muscle cells, eosinophils, synovial membranes in rheumatoid arthritis, dermal fibroblasts, and other non-lymphoid cell types. Depending on the type of cell expressing CD40, ligation can induce intercellular adhesion, differentiation, activation, and proliferation. For example, binding of CD40 to its cognate ligand, CD40L (also designated CD154),  
15 stimulates B-cell proliferation and differentiation into plasma cells, antibody production, isotype switching, and B-cell memory generation. During B-cell differentiation, CD40 is expressed on pre-B cells but lost upon differentiation into plasma cells.

The CD40 ligand has been identified on the cell surface of activated T cells (Fenslow *et al.* (1992) *J. Immunol.* 149:655; Lane *et al.* (1992) *Eur. J. Immunol.* 22:2573; Noelle *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6550), but is not generally expressed  
20 on resting human T cells. CD40L is a type-II transmembrane glycoprotein with homology to TNF- $\alpha$  (Armitage *et al.* (1992) *Nature* 357:80 and Spriggs *et al.* (1992) *J. Exp. Med.* 176:1543). The extracellular domain of CD40L contains two arginine residues

proximal to the transmembrane region, providing a potential proteolytic cleavage site that gives rise to a soluble form of the ligand (sCD40L). Overexpression of CD40L causes autoimmune diseases similar to systemic lupus erythematosus in rodent models (Higuchi *et al.* (2002) *J. Immunol.* 168:9-12). In contrast, absence of functional CD40L on  
5 activated T cells causes X-linked hyper-IgM syndrome (Allen *et al.* (1993) *Science* 259:990; and Korthauer *et al.* (1993) *Nature* 361:539). Further, blocking of CD40/CD40L interaction can prevent transplant rejection in non-human primate models. See, for example, Wee *et al.* (1992) *Transplantation* 53:501-7.

CD40 expression on APCs plays an important co-stimulatory role in the  
10 activation of these cells. For example, agonistic anti-CD40 monoclonal antibodies (mAbs) have been shown to mimic the effects of T helper cells in B-cell activation. When presented on adherent cells expressing FcγRII, these antibodies induce B-cell proliferation (Banchereau *et al.* (1989) *Science* 251:70). Moreover, agonistic anti-CD40 mAbs can replace the T helper signal for secretion of IgM, IgG, and IgE in the presence  
15 of IL-4 (Gascan *et al.* (1991) *J. Immunol.* 147:8). Furthermore, agonistic anti-CD40 mAbs can prevent programmed cell death (apoptosis) of B cells isolated from lymph nodes.

These and other observations support the current theory that the interaction of CD40 and CD40L plays a pivotal role in regulating both humoral and cell-mediated  
20 immune responses. More recent studies have revealed a much broader role of CD40/CD40L interaction in diverse physiological and pathological processes.

The CD40 signal transduction pathway depends on the coordinated regulation of many intracellular factors. Like other members of the TNF receptor family, CD40 reacts with TRAF proteins (TNF receptor factor-associated proteins) such as TRAF2 and  
25 TRAF3, which mediate an intracellular signal following engagement of CD40 with CD40L (either solid phase CD40L or soluble CD40L). The TRAFs transduce a signal into the nucleus via map kinases such as NIK (NF-κB inducing kinase) and I-kappa B kinases (IKK α/β), ultimately activating the transcription factor NF-κB (Young *et al.* (1998) *Immunol. Today* 19:502-06). Signaling via Ras and the MEK/ERK pathway has  
30 also been demonstrated in a subset of B cells. Additional pathways involved in CD40

cell signaling include PI3K/Akt pathway and P38 MAPK pathway (Craxton *et al.* (1998) *J. Immunol.* 5:439-447).

5 Signaling via CD40 has been shown to prevent cell death from apoptosis (Makus *et al.* (2002) *J. Immunol.* 14:973-982). Apoptotic signals are necessary to induce programmed cell death in a coordinated manner. Cell death signals can include intrinsic stimuli from within the cell such as endoplasmic reticulum stress or extrinsic stimuli such as receptor binding of FasL or TNF $\alpha$ . The signaling pathway is complex, involving activation of caspases such as caspase 3 and 9, and of poly (ADP ribose) polymerase (PARP). During the cascade, anti-apoptotic signaling proteins, such as Mcl-1 and BCLx, and members of the IAP-family proteins, such as X-Linked Inhibitor of Apoptosis (XIAP), are down-regulated (Budihardjo *et al.* (1999) *Annu. Rev. Cell Dev. Biol.* 15:269-90). For example, in dendritic cells, CD40 cell signaling can block apoptosis signals transduced by FasL (Bjorck *et al.* (1997) *Int'l Immunol.* 9:365-372).

15 Thus, CD40 engagement by CD40L and subsequent activation of CD40 signaling are necessary steps for normal immune responses; however, dysregulation of CD40 signaling can lead to disease. The CD40 signaling pathway has been shown to be involved autoimmune disease (Ichikawa *et al.* (2002) *J. Immunol.* 169:2781-7 and Moore *et al.* (2002) *J. Autoimmun.* 19:139-45). Additionally, the CD40/CD40L interaction plays an important role in inflammatory processes. For example, both CD40 and CD40 ligand are overexpressed in human and experimental atherosclerosis lesions. CD40 stimulation induces expression of matrix-degrading enzymes and tissue factor expression in atheroma-associated cell types, such as endothelial cells, smooth muscle cells, and macrophages. Further, CD40 stimulation induces production of proinflammatory cytokines such as IL-1, IL-6, and IL-8, and adhesion molecules such as ICAM-1, E-selectin, and VCAM. Inhibition of CD40/CD40L interaction prevents atherogenesis in animal models. In transplant models, blocking CD40/CD40L interaction prevents inflammation. It has been shown that CD40/CD40L binding acts synergistically with the Alzheimer amyloid-beta peptide to promote microglial activation, thus leading to neurotoxicity.

30 In patients with rheumatoid arthritis (RA), CD40 expression is increased on articular chondrocytes, thus, CD40 signaling likely contributes to production of damaging

cytokines and matrix metalloproteinases. See, Gotoh *et al.* (2004) *J. Rheumatol.* 31:1506-12. Further, it has been shown that amplification of the synovial inflammatory response occurs through activation of mitogen-activated protein (MAP) kinases and nuclear factor kappaB (NFκB) via ligation of CD40 on CD14<sup>+</sup> synovial cells from RA patients (Harigai *et al.* (2004) *Arthritis. Rheum.* 50:2167-77). In an experimental model of RA, anti-CD40L antibody treatment prevented disease induction, joint inflammation, and anti-collagen antibody production (Durie *et al.* (1993) *Science* 261:1328). Finally, in clinical trials, it has been shown that depleting CD20<sup>+</sup> positive B cells of RA patients by administering RITUXAN® (generally indicated for B cell lymphoma) improves symptoms. (Shaw *et al.* (2003) *Ann. Rheum. Dis.* 62:ii55-ii59).

Blocking CD40/CD40L interactions during antigen presentation to T cells has also been shown to induce T cell tolerance. Therefore, blocking CD40/CD40L interaction prevents initial T cell activation as well as induces long term tolerance to re-exposure to the antigen.

Given the critical role of CD40L-mediated CD40 signaling in maintenance of normal immunity, methods are needed for intervention into this signaling pathway when dysregulation occurs.

## BRIEF SUMMARY OF THE INVENTION

Methods are provided for treating a human subject with an autoimmune disease and/or inflammatory disease, comprising administering to the subject an anti-CD40 antibody or an antigen-binding fragment thereof that is free of significant agonist activity  
5 when bound to a CD40 antigen on a human CD40-expressing cell. Methods for inhibiting the response of cells expressing CD40 antigen are also provided. Suitable antagonist anti-CD40 antibodies for use in the methods of the present invention have a strong affinity for CD40. These monoclonal antibodies and antigen-binding fragments thereof are capable of specifically binding to human CD40 antigen expressed on the  
10 surface of a human cell. They are free of significant agonist activity but exhibit antagonist activity when bound to CD40 antigen on human cells. In one embodiment, the anti-CD40 antibody or fragment thereof exhibits antagonist activity when bound to CD40 antigen on antigen presenting cells such as B cells.

The antagonist antibodies are especially useful in preventing, ameliorating, or  
15 treating diseases comprising an autoimmune and/or inflammatory component. These diseases include but are not limited to autoimmune and inflammatory diseases such as systemic lupus erythematosus (SLE), discoid lupus, lupus nephritis, sarcoidosis, inflammatory arthritis, including, but not limited to, juvenile arthritis, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, and gouty arthritis,  
20 rejection of an organ or tissue transplant, hyperacute, acute, or chronic rejection and/or graft versus host disease, multiple sclerosis, hyper IgE syndrome, polyarteritis nodosa, primary biliary cirrhosis, inflammatory bowel disease, Crohn's disease, celiac's disease (gluten-sensitive enteropathy), autoimmune hepatitis, pernicious anemia, autoimmune hemolytic anemia, psoriasis, scleroderma, myasthenia gravis, autoimmune  
25 thrombocytopenic purpura, autoimmune thyroiditis, Grave's disease, Hashimoto's thyroiditis, immune complex disease, chronic fatigue immune dysfunction syndrome (CFIDS), polymyositis and dermatomyositis, cryoglobulinemia, thrombolysis, cardiomyopathy, pemphigus vulgaris, pulmonary interstitial fibrosis, sarcoidosis, Type I and Type II diabetes mellitus, type 1, 2, 3, and 4 delayed-type hypersensitivity, allergy or  
30 allergic disorders, unwanted/unintended immune responses to therapeutic proteins, asthma, Churg-Strauss syndrome (allergic granulomatosis), atopic dermatitis, allergic and

irritant contact dermatitis, urticaria, IgE-mediated allergy, atherosclerosis, vasculitis, idiopathic inflammatory myopathies, hemolytic disease, Alzheimer's disease, chronic inflammatory demyelinating polyneuropathy, and the like.

5

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 sets forth the amino acid sequences for the light and heavy chains of the mAb CHIR-12.12. The leader (residues 1-20 of SEQ ID NO:2), variable (residues 21-132 of SEQ ID NO:2), and constant (residues 133-239 of SEQ ID NO:2) regions of the light chain are shown in Figure 1A. The leader (residues 1-19 of SEQ ID NO:4), variable (residues 20-139 of SEQ ID NO:4), and constant (residues 140-469 of SEQ ID NO:4) regions of the heavy chain are shown in Figure 1B. The alternative constant region for the heavy chain of the mAb CHIR-12.12 shown in Figure 1B reflects a substitution of a serine residue for the alanine residue at position 153 of SEQ ID NO:4. The complete sequence for this variant of the heavy chain of the mAb CHIR-12.12 is set forth in SEQ ID NO:5.

15

Figure 2 shows the coding sequence for the light chain (Figure 2A; SEQ ID NO:1) and heavy chain (Figure 2B; SEQ ID NO:3) for the mAb CHIR-12.12.

Figure 3 sets forth the amino acid sequences for the light and heavy chains of mAb CHIR-5.9. The leader (residues 1-20 of SEQ ID NO:6), variable (residues 21-132 of SEQ ID NO:6), and constant (residues 133-239 of SEQ ID NO:6) regions of the light chain are shown in Figure 3A. The leader (residues 1-19 of SEQ ID NO:7), variable (residues 20-144 of SEQ ID NO:7), and constant (residues 145-474 of SEQ ID NO:7) regions of the heavy chain are shown in Figure 3B. The alternative constant region for the heavy chain of the mAb CHIR-5.9 shown in Figure 3B reflects a substitution of a serine residue for the alanine residue at position 158 of SEQ ID NO:7. The complete sequence for this variant of the heavy chain of the mAb CHIR-5.9 is set forth in SEQ ID NO:8.

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Figure 4 shows the coding sequence (Figure 4A; SEQ ID NO:9) for the short isoform of human CD40 (amino acid sequence shown in Figure 4B; SEQ ID NO:10), and the coding sequence (Figure 4C; SEQ ID NO:11) for the long isoform of human CD40 (amino acid sequence shown in Figure 4D).

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Figure 5 shows thermal melting temperature of CHIR-12.12 in different pH formulations measured by differential scanning calorimetry (DSC).

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## DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to methods for treating autoimmune and inflammatory diseases, using antibodies having a strong affinity for the CD40 cell surface antigen. These anti-CD40 antibodies and antigen-binding fragments thereof are free of significant agonist activity and exhibit antagonist activity when bound to CD40 on CD40-expressing cells.

"Antibodies" and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to an antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

The term "antibody" is used in the broadest sense and covers fully assembled antibodies, antibody fragments that can bind antigen (e.g., Fab', F'(ab)<sub>2</sub>, Fv, single chain antibodies, diabodies), and recombinant peptides comprising the foregoing.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V<sub>H</sub>) followed by a number of constant domains. Each light chain has a variable domain at one end (V<sub>L</sub>) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light



chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains  
5 differ extensively in sequence among antibodies and are used in the binding and  
specificity of each particular antibody for its particular antigen. However, the variability  
is not evenly distributed throughout the variable domains of antibodies. It is concentrated  
in three segments called complementarity determining regions (CDRs) or hypervariable  
regions both in the light-chain and the heavy-chain variable domains. The more highly  
10 conserved portions of variable domains are called the framework (FR) regions. The  
variable domains of native heavy and light chains each comprise four FR regions, largely  
adopting a  $\beta$ -sheet configuration, connected by three CDRs, which form loops  
connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs in each  
chain are held together in close proximity by the FR regions and, with the CDRs from the  
15 other chain, contribute to the formation of the antigen-binding site of antibodies (see  
Kabat *et al.* (1991) *NIH Publ. No. 91-3242*, Vol. I, pages 647-669).

The constant domains are not involved directly in binding an antibody to an  
antigen, but exhibit various effector functions, such as Fc receptor (FcR) binding,  
participation of the antibody in antibody-dependent cellular toxicity, opsonization,  
20 initiation of complement dependent cytotoxicity, and mast cell degranulation.

The term "hypervariable region" when used herein refers to the amino acid  
residues of an antibody that are responsible for antigen binding. The hypervariable  
region comprises amino acid residues from a "complementarity determining region" or  
"CDR" (i.e., residues 24-34 (L1), 50-56 (L2), and 89-97 (L3) in the light-chain variable  
25 domain and 31-35 (H1), 50-65 (H2), and 95-102 (H3) in the heavy-chain variable  
domain; Kabat *et al.* (1991) *Sequences of Proteins of Immunological Interest* (5th ed.,  
Public Health Service, National Institute of Health, Bethesda, MD) and/or those residues  
from a "hypervariable loop" (i.e., residues 26-32 (L1), 50-52 (L2), and 91-96 (L3) in the  
light-chain variable domain and 26-32 (H1), 53-55 (H2), and 96-101 (H3) in the heavy-  
30 chain variable domain; Clothia and Lesk (1987) *J. Mol. Biol.* 196:901-917).

"Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody  
5 fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (Zapata *et al.* (1995) *Protein Eng.* 8(10):1057-1062); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name  
10 reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment that contains a complete antigen recognition and binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a  
15 single-chain Fv species, one heavy- and one light-chain variable domain can be covalently linked by flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V<sub>H</sub>-V<sub>L</sub> dimer. Collectively, the six CDRs confer  
20 antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (C<sub>H</sub>1) of the heavy chain. Fab fragments differ from Fab' fragments by  
25 the addition of a few residues at the carboxy terminus of the heavy-chain C<sub>H</sub>1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody  
30 fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of human immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Different isotypes have different effector functions. For example, human IgG1 and IgG3 isotypes mediate antibody-dependent cell-mediated cytotoxicity (ADCC) activity.

The word "label" when used herein refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native target disclosed herein or the transcription or translation thereof.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, succinate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or

sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN, polyethylene glycol (PEG), and Pluronic. Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

5           A "host cell," as used herein, refers to a microorganism or a eukaryotic cell or cell line cultured as a unicellular entity that can be, or has been, used as a recipient for a recombinant vector or other transfer polynucleotides, and include the progeny of the original cell that has been transfected. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA  
10 complement as the original parent, due to natural, accidental, or deliberate mutation.

          "Human effector cells" are leukocytes that express one or more FcRs and perform effector functions. Preferably, the cells express at least Fc $\gamma$ RIII and carry out antigen-dependent cell-mediated cytotoxicity (ADCC) effector function. Examples of human leukocytes that mediate ADCC include peripheral blood mononuclear cells (PBMC),  
15 natural killer (NK) cells, monocytes, macrophages, eosinophils, and neutrophils, with PBMCs and NK cells being preferred. Antibodies that have ADCC activity are typically of the IgG1 or IgG3 isotype. Note that in addition to isolating IgG1 and IgG3 antibodies, such ADCC-mediating antibodies can be made by engineering a variable region from a non-ADCC antibody or variable region fragment to an IgG1 or IgG3 isotype constant  
20 region.

          The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native-sequence human FcR. Moreover, a preferred FcR is one that binds an IgG antibody (a gamma receptor) and includes receptors of the Fc $\gamma$ RI, Fc $\gamma$ RII, and Fc $\gamma$ RIII subclasses, including allelic variants and  
25 alternatively spliced forms of these receptors. Fc $\gamma$ RII receptors include Fc $\gamma$ RIIA (an "activating receptor") and Fc $\gamma$ RIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor Fc $\gamma$ RIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor Fc $\gamma$ RIIB contains an immunoreceptor  
30 tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (see Daeron (1997) *Annu. Rev. Immunol.* 15:203-234). FcRs are reviewed in Ravetch and Kinet (1991)

*Annu. Rev. Immunol.* 9:457-492 (1991); Capel *et al.* (1994) *Immunomethods* 4:25-34; and de Haas *et al.* (1995) *J. Lab. Clin. Med.* 126:330-341. Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.* (1976) *J. Immunol.* 117:587 and Kim *et al.* (1994) *J. Immunol.* 24:249 (1994)).

There are a number of ways to make human antibodies. For example, secreting cells can be immortalized by infection with the Epstein-Barr virus (EBV). However, EBV-infected cells are difficult to clone and usually produce only relatively low yields of immunoglobulin (James and Bell (1987) *J. Immunol. Methods* 100:5-40). In the future, the immortalization of human B cells might possibly be achieved by introducing a defined combination of transforming genes. Such a possibility is highlighted by a recent demonstration that the expression of the telomerase catalytic subunit together with the SV40 large oncoprotein and an oncogenic allele of H-ras resulted in the tumorigenic conversion of normal human epithelial and fibroblast cells (Hahn *et al.* (1999) *Nature* 400:464-468). It is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production (Jakobovits *et al.* (1993) *Nature* 362:255-258; Lonberg and Huszar (1995) *Int. Rev. Immunol.* 13:65-93; Fishwild *et al.* (1996) *Nat. Biotechnol.* 14:845-851; Mendez *et al.* (1997) *Nat. Genet.* 15:146-156; Green (1999) *J. Immunol. Methods* 231:11-23; Tomizuka *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:722-727; reviewed in Little *et al.* (2000) *Immunol. Today* 21:364-370). For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J<sub>H</sub>) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production (Jakobovits *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:2551-2555). Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice results in the production of human antibodies upon antigen challenge (Jakobovits *et al.* (1993) *Nature* 362:255-258). Mendez *et al.* (1997) (*Nature Genetics* 15:146-156) have generated a line of transgenic mice that, when challenged with an antigen, generates high affinity fully human antibodies. This was achieved by germ-line integration of megabase human heavy-chain and light-chain loci into mice with

deletion into endogenous J<sub>H</sub> segment as described above. These mice (XenoMouse<sup>®</sup> II technology (Abgenix; Fremont, California)) harbor 1,020 kb of human heavy-chain locus containing approximately 66 V<sub>H</sub> genes, complete D<sub>H</sub> and J<sub>H</sub> regions, and three different constant regions, and also harbors 800 kb of human  $\kappa$  locus containing 32 V $\kappa$  genes, J $\kappa$  segments, and C $\kappa$  genes. The antibodies produced in these mice closely resemble that seen in humans in all respects, including gene rearrangement, assembly, and repertoire. The human antibodies are preferentially expressed over endogenous antibodies due to deletion in endogenous segment that prevents gene rearrangement in the murine locus. Such mice may be immunized with an antigen of particular interest.

Sera from such immunized animals may be screened for antibody reactivity against the initial antigen. Lymphocytes may be isolated from lymph nodes or spleen cells and may further be selected for B cells by selecting for CD138-negative and CD19-positive cells. In one aspect, such B cell cultures (BCCs) may be fused to myeloma cells to generate hybridomas as detailed above.

In another aspect, such B cell cultures may be screened further for reactivity against the initial antigen, preferably. Such screening includes ELISA with the target/antigen protein, a competition assay with known antibodies that bind the antigen of interest, and *in vitro* binding to transiently transfected CHO or other cells that express the target antigen.

The present invention is directed to compositions and methods for treating human subjects having autoimmune diseases and/or inflammatory diseases. The methods involve treatment with an anti-CD40 antibody described herein, or an antigen-binding fragment thereof, where administration of the antibody or antigen-binding fragment thereof promotes a positive therapeutic response within the subject undergoing this method of therapy. The methods and compositions are especially useful in treating diseases that include, but are not limited to, autoimmune diseases such as systemic lupus erythematosus (SLE), discoid lupus, lupus nephritis, sarcoidosis, inflammatory arthritis, including juvenile arthritis, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, and gouty arthritis, rejection of an organ or tissue transplant, hyperacute, acute, or chronic rejection and/or graft versus host disease, multiple sclerosis, hyper IgE syndrome, polyarteritis nodosa, primary biliary cirrhosis, inflammatory bowel

disease, Crohn's disease, celiac's disease (gluten-sensitive enteropathy), autoimmune hepatitis, pernicious anemia, autoimmune hemolytic anemia, psoriasis, scleroderma, myasthenia gravis, autoimmune thrombocytopenic purpura, autoimmune thyroiditis, Grave's disease, Hashimoto's thyroiditis, immune complex disease, chronic fatigue

5 immune dysfunction syndrome (CFIDS), polymyositis and dermatomyositis, cryoglobulinemia, thrombolysis, cardiomyopathy, pemphigus vulgaris, pulmonary interstitial fibrosis, Type I and Type II diabetes mellitus, and the like. Additionally, these antagonist anti-CD40 antibodies and antigen-binding fragments thereof are especially useful in treating diseases associated with inflammation, including, but not limited to,

10 type 1, 2, 3, and 4 delayed-type hypersensitivity, allergy or allergic disorders, unwanted/unintended immune responses to therapeutic proteins (see for example, U.S. Patent Application No. US 2002/0119151 and Koren, *et al.* (2002) *Curr. Pharm. Biotechnol.* 3:349-60), asthma, Churg-Strauss syndrome (allergic granulomatosis), atopic dermatitis, allergic and irritant contact dermatitis, urticaria, IgE-mediated allergy,

15 atherosclerosis, vasculitis, idiopathic inflammatory myopathies, hemolytic disease, Alzheimer's disease, chronic inflammatory demyelinating polyneuropathy, and the like.

Anti-CD40 antibodies suitable for use in the methods of the invention specifically bind a human CD40 antigen expressed on the surface of a human cell and are free of significant agonist activity, but exhibit antagonist activity when bound to the CD40

20 antigen on a human CD40-expressing cell. These anti-CD40 antibodies and antigen-binding fragments thereof are referred to herein as "antagonist anti-CD40 antibodies." Such antibodies include, but are not limited to, the fully human monoclonal antibodies CHIR-5.9 and CHIR-12.12 described below and monoclonal antibodies having the binding characteristics of monoclonal antibodies CHIR-5.9 and CHIR-12.12, also

25 described below. These monoclonal antibodies, which can be recombinantly produced, are discussed below and disclosed in the copending provisional applications entitled "*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*," filed November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)),

30 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710

(Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety.

Antibodies that have the binding characteristics of monoclonal antibodies CHIR-5.9 and CHIR-12.12 include antibodies that competitively interfere with binding CD40 and/or bind the same epitopes as CHIR-5.9 and CHIR-12.12. One of skill in the art could determine whether an antibody competitively interferes with CHIR-5.9 or CHIR-12.12 using standard methods known in the art.

When these antibodies bind CD40 displayed on the surface of human cells, such as, for example, human B cells, T cells, dendritic cells, endothelial cells, activated platelets, inflamed vascular smooth muscle cells, eosinophils, synovial membranes, dermal fibroblasts, and the like, the antibodies are free of significant agonist activity. In some embodiments, their binding to CD40 displayed on the surface of human cells results in inhibition of activation and differentiation of these human cells. Thus, the antagonist anti-CD40 antibodies suitable for use in the methods of the invention include those monoclonal antibodies that can exhibit antagonist activity toward normal and abnormal human cells expressing the cell-surface CD40 antigen.

#### Antagonist Anti-CD40 Antibodies

The monoclonal antibodies CHIR-5.9 and CHIR-12.12 represent suitable antagonist anti-CD40 antibodies for use in the methods of the present invention. The CHIR-5.9 and CHIR-12.12 antibodies are fully human anti-CD40 monoclonal antibodies of the IgG<sub>1</sub> isotype produced from the hybridoma cell lines 131.2F8.5.9 (referred to herein as the cell line 5.9) and 153.8E2.D10.D6.12.12 (referred to herein as the cell line 12.12). These cell lines were created using splenocytes from immunized xenotypic mice containing the human IgG<sub>1</sub> heavy chain locus and the human K chain locus (XenoMouse<sup>®</sup> technology (Abgenix; Fremont, California)). The spleen cells were fused with the mouse myeloma SP2/0 cells (Sierra BioSource). The resulting hybridomas were sub-cloned several times to create the stable monoclonal cell lines 5.9 and 12.12. Other antibodies of the invention may be prepared similarly using mice transgenic for human immunoglobulin loci or by other methods known in the art and/or described herein.



The nucleotide and amino acid sequences of the variable regions of the CHIR-12.12 antibody, and the amino acid sequences of the variable regions of the CHIR-5.9 antibody, are disclosed in copending provisional applications entitled "*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*," filed November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)), 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710 (Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety. The amino acid sequences for the leader, variable, and constant regions for the light chain and heavy chain for mAb CHIR-12.12 are set forth herein in Figures 1A and 1B, respectively. See also SEQ ID NO:2 (complete sequence for the light chain of mAb CHIR-12.12), SEQ ID NO:4 (complete sequence for the heavy chain for mAb CHIR-12.12), and SEQ ID NO:5 (complete sequence for a variant of the heavy chain for mAb CHIR-12.12 set forth in SEQ ID NO:4, where the variant comprises a serine substitution for the alanine residue at position 153 of SEQ ID NO:4). The nucleotide sequences encoding the light chain and heavy chain for mAb CHIR-12.12 are set forth herein in Figures 2A and 2B, respectively. See also SEQ ID NO:1 (coding sequence for the light chain for mAb CHIR-12.12), and SEQ ID NO:3 (coding sequence for the heavy chain for mAb CHIR-12.12). The amino acid sequences for the leader, variable, and constant regions for the light chain and heavy chain of the CHIR-5.9 mAb are set forth herein in Figures 3A and 3B, respectively. See also SEQ ID NO:6 (complete sequence for the light chain of mAb CHIR-5.9), SEQ ID NO:7 (complete sequence for the heavy chain of mAb CHIR-5.9), and SEQ ID NO:8 (complete sequence for a variant of the heavy chain of mAb CHIR-5.9 set forth in SEQ ID NO:7, where the variant comprises a serine substitution for the alanine residue at position 158 of SEQ ID NO:7). Further, hybridomas expressing CHIR-5.9 and CHIR-12.12 antibodies have been deposited with the ATCC with a patent deposit designation of PTA-5542 and PTA-5543, respectively.

The CHIR-5.9 and CHIR-12.12 monoclonal antibodies bind soluble CD40 in ELISA-type assays, prevent the binding of CD40-ligand to cell-surface CD40, and displace the pre-bound CD40-ligand, as determined by flow cytometric assays.

Antibodies CHIR-5.9 and CHIR-12.12 compete with each other for binding to CD40 but not with 15B8, the anti-CD40 monoclonal antibody described in U.S. Provisional Application Serial No. 60/237,556, titled "*Human Anti-CD40 Antibodies*," filed October 2, 2000, and PCT International Application No. PCT/US01/30857, also titled "*Human Anti-CD40 Antibodies*," filed October 2, 2001 (Attorney Docket No. PP16092.003), both of which are herein incorporated by reference in their entirety. When tested *in vitro* for effects on proliferation of B cells from normal human subjects, CHIR-5.9 and CHIR-12.12 act as antagonistic anti-CD40 antibodies. Furthermore, CHIR-5.9 and CHIR-12.12 do not induce strong proliferation of human lymphocytes from normal subjects. The binding affinity of CHIR-5.9 for human CD40 is  $1.2 \times 10^{-8}$  M and the binding affinity of CHIR-12.12 is  $5 \times 10^{-10}$  M, as determined by the Biacore™ assay.

Suitable antagonist anti-CD40 antibodies for use in the methods of the present invention exhibit a strong single-site binding affinity for the CD40 cell-surface antigen. The monoclonal antibodies of the invention exhibit a dissociation equilibrium constant ( $K_D$ ) for CD40 of at least  $10^{-5}$  M, at least  $3 \times 10^{-5}$  M, preferably at least  $10^{-6}$  M to  $10^{-7}$  M, more preferably at least  $10^{-8}$  M to about  $10^{-12}$  M, measured using a standard assay such as Biacore™. Biacore analysis is known in the art and details are provided in the "BIAapplications handbook." Methods described in WO 01/27160 can be used to modulate the binding affinity.

By "CD40 antigen," "CD40 cell surface antigen," "CD40 receptor," or "CD40" is intended a transmembrane glycoprotein that belongs to the tumor necrosis factor (TNF) receptor family (see, for example, U.S. Patent Nos. 5,674,492 and 4,708,871; Stamenkovic *et al.* (1989) *EMBO* 8:1403; Clark (1990) *Tissue Antigens* 36:33; Barclay *et al.* (1997) *The Leucocyte Antigen Facts Book* (2d ed.; Academic Press, San Diego)). Two isoforms of human CD40, encoded by alternatively spliced transcript variants of this gene, have been identified. The first isoform (also known as the "long isoform" or "isoform 1") is expressed as a 277-amino-acid precursor polypeptide (SEQ ID NO:12 (first reported as GenBank Accession No. CAA43045, and identified as isoform 1 in GenBank Accession No. NP\_001241), encoded by SEQ ID NO:11 (see GenBank Accession Nos. X60592 and NM\_001250)), which has a signal sequence represented by the first 19 residues. The second isoform (also known as the "short isoform" or "isoform

2") is expressed as a 203-amino-acid precursor polypeptide (SEQ ID NO:10 (GenBank Accession No. NP\_690593), encoded by SEQ ID NO:9 (GenBank Accession No. NM\_152854)), which also has a signal sequence represented by the first 19 residues. The precursor polypeptides of these two isoforms of human CD40 share in common their first  
5 165 residues (i.e., residues 1-165 of SEQ ID NO:10 and SEQ ID NO:12). The precursor polypeptide of the short isoform (shown in SEQ ID NO:10) is encoded by a transcript variant (SEQ ID NO:9) that lacks a coding segment, which leads to a translation frame shift; the resulting CD40 isoform contains a shorter and distinct C-terminus (residues 166-203 of SEQ ID NO:10) from that contained in the long isoform of CD40 (C-terminus  
10 shown in residues 166-277 of SEQ ID NO:12). For purposes of the present invention, the term "CD40 antigen," "CD40 cell surface antigen," "CD40 receptor," or "CD40" encompasses both the short and long isoforms of CD40. The anti-CD40 antibodies of the present invention bind to an epitope of human CD40 that resides at the same location within either the short isoform or long isoform of this cell surface antigen as noted herein  
15 below.

The CD40 antigen is displayed on the surface of a variety of cell types, as described elsewhere herein. By "displayed on the surface" and "expressed on the surface" is intended that all or a portion of the CD40 antigen is exposed to the exterior of the cell. The displayed or expressed CD40 antigen may be fully or partially glycosylated.

20 By "agonist activity" is intended that the substance functions as an agonist. An agonist combines with a receptor on a cell and initiates a reaction or activity that is similar to or the same as that initiated by the receptor's natural ligand. For example, an agonist of CD40 induces any or all of, but not limited to, the following responses: cell proliferation and/or differentiation; upregulation of intercellular adhesion via such  
25 molecules as ICAM-1, E-selectin, VCAM, and the like; secretion of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12, TNF, and the like; signal transduction through the CD40 receptor by such pathways as TRAF (e.g., TRAF2 and/or TRAF3), MAP kinases such as NIK (NF- $\kappa$ B inducing kinase), I-kappa B kinases (IKK  $\alpha/\beta$ ), transcription factor NF- $\kappa$ B, Ras and the MEK/ERK pathway, the PI3K/Akt pathway, the  
30 P38 MAPK pathway, and the like; transduction of an anti-apoptotic signal by such molecules as XIAP, Mcl-1, BCLx, and the like; B and/or T cell memory generation; B

cell antibody production; B cell isotype switching, up-regulation of cell-surface expression of MHC Class II and CD80/86, and the like. By "antagonist activity" is intended that the substance functions as an antagonist. For example, an antagonist of CD40 prevents or reduces induction of any of the responses induced by binding of the CD40 receptor to an agonist ligand, particularly CD40L. The antagonist may reduce induction of any one or more of the responses to agonist binding by 5%, 10%, 15%, 20%, 25%, 30%, 35%, preferably 40%, 45%, 50%, 55%, 60%, more preferably 70%, 80%, 85%, and most preferably 90%, 95%, 99%, or 100%. Methods for measuring anti-CD40 antibody and CD40-ligand binding specificity and antagonist activity are known to one of skill in the art and include, but are not limited to, standard competitive binding assays, assays for monitoring immunoglobulin secretion by B cells, B cell proliferation assays, Banchereau-Like-B cell proliferation assays, T cell helper assays for antibody production, co-stimulation of B cell proliferation assays, and assays for up-regulation of B cell activation markers. See, for example, such assays disclosed in WO 00/75348, U.S. Patent No. 6,087,329, and copending provisional applications entitled "*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*," filed November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)), 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710 (Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety.

By "significant" agonist activity is intended an agonist activity of at least 30%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than the agonist activity induced by a neutral substance or negative control as measured in a bioassay such as a B cell response assay. Preferably, "significant" agonist activity is an agonist activity that is at least 2-fold greater or at least 3-fold greater than the agonist activity induced by a neutral substance or negative control as measured in a bioassay such as a B cell response assay. Thus, for example, where a B cell response is of interest a B cell proliferation assay is used, and "significant" agonist activity would be induction of a level of B cell proliferation that is at least 2-fold greater or at least 3-fold greater than the level of B cell proliferation induced by a neutral substance or negative control. In one

embodiment, a non-specific immunoglobulin, for example IgG1, that does not bind to CD40 serves as the negative control. A substance "free of significant agonist activity" would exhibit an agonist activity of not more than about 25% greater than the agonist activity induced by a neutral substance or negative control, preferably not more than about 20% greater, 15% greater, 10% greater, 5% greater, 1% greater, 0.5% greater, or even not more than about 0.1% greater than the agonist activity induced by a neutral substance or negative control as measured in a bioassay such as a B cell response assay. The antagonist anti-CD40 antibodies useful in the methods of the present invention are free of significant agonist activity as noted above when bound to a CD40 antigen on a human cell. In one embodiment of the invention, the antagonist anti-CD40 antibody is free of significant agonist activity in one cellular response. In another embodiment of the invention, the antagonist anti-CD40 antibody is free of significant agonist activity in assays of more than one cellular response (e.g., proliferation and differentiation, or proliferation, differentiation, and, for B cells, antibody production).

As used herein "anti-CD40 antibody" encompasses any antibody that specifically recognizes the CD40 antigen, including polyclonal antibodies, monoclonal antibodies, single-chain antibodies, and fragments thereof such as Fab, F(ab')<sub>2</sub>, F<sub>v</sub>, and other fragments which retain the antigen binding function of the parent anti-CD40 antibody. Of particular interest to the methods of the present invention are antagonist anti-CD40 antibodies that share the binding characteristics of the monoclonal antibodies CHIR-5.9 and CHIR-12.12 described above.

Thus, in addition to the monoclonal antibodies CHIR-5.9 and CHIR-12.12, other antibodies that would be useful in practicing the methods of the invention described herein include, but are not limited to, the following: (1) the monoclonal antibodies produced by the hybridoma cell lines designated 131.2F8.5.9 (referred to herein as the cell line 5.9) and 153.8E2.D10.D6.12.12 (referred to herein as the cell line 12.12), deposited with the ATCC as Patent Deposit No. PTA-5542 and Patent Deposit No. PTA-5543, respectively; (2) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequences shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequences shown in SEQ ID NO:2 and

SEQ ID NO:5; (3) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequences shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequences shown in SEQ ID NO:6 and SEQ ID NO:8; (4) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence shown in SEQ ID NO:1, the nucleotide sequence shown in SEQ ID NO:3, and both the sequences shown in SEQ ID NO:1 and SEQ ID NO:3; (5) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 5.9 or the hybridoma cell line 12.12; (6) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the amino acid sequence shown in SEQ ID NO:10 or SEQ ID NO:12; (7) a monoclonal antibody that competes with the monoclonal antibody CHIR-5.9 or CHIR-12.12 in a competitive binding assay; and (8) a monoclonal antibody that is an antigen-binding fragment of the CHIR-12.12 or CHIR-5.9 monoclonal antibody or the foregoing monoclonal antibodies in preceding items (1)-(7), where the fragment retains the capability of specifically binding to the human CD40 antigen. Those skilled in the art recognize that the antagonist anti-CD40 antibodies and antigen-binding fragments of these antibodies suitable for use in the methods disclosed herein include antagonist anti-CD40 antibodies and antigen-binding fragments thereof that are produced recombinantly using methods well known in the art and described herein below, and include, for example, monoclonal antibodies CHIR-5.9 and CHIR-12.12 that have been recombinantly produced.

## Production of Anti-CD40 Antibodies

The antagonist anti-CD40 antibodies for use in the methods of the present invention can be produced using any of the methods well known to those of skill in the art. Polyclonal sera may be prepared by conventional methods. In general, a solution containing the CD40 antigen is first used to immunize a suitable animal, preferably a mouse, rat, rabbit, or goat. Rabbits or goats are preferred for the preparation of

polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies.

Polyclonal sera can be prepared in a transgenic animal, preferably a mouse bearing human immunoglobulin loci. In a preferred embodiment, Sf9 cells expressing CD40 are used as the immunogen. Immunization can also be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization. Polyclonal antisera are obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000 x g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Production of the Sf 9 (*Spodoptera frugiperda*) cells is disclosed in U.S. Patent No. 6,004,552, incorporated herein by reference. Briefly, sequences encoding human CD40 were recombined into a baculovirus using transfer vectors. The plasmids were co-transfected with wild-type baculovirus DNA into Sf 9 cells. Recombinant baculovirus-infected Sf 9 cells were identified and clonally purified.

Preferably the antibody is monoclonal in nature. By "monoclonal antibody" is intended an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The term is not limited regarding the species or source of the antibody. The term encompasses whole immunoglobulins as well as fragments such as Fab, F(ab')<sub>2</sub>, Fv, and others which retain the antigen binding function of the antibody. Monoclonal antibodies are highly specific, being directed against a single antigenic site, i.e., the CD40 cell surface antigen in the present invention. Furthermore, in contrast to conventional (polyclonal) antibody preparations that typically include different antibodies directed

against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in, for example, Clackson *et al.* (1991) *Nature* 352:624-628; Marks *et al.* (1991) *J. Mol. Biol.* 222:581-597; and U.S. Patent No. 5,514,548.

By "epitope" is intended the part of an antigenic molecule to which an antibody is produced and to which the antibody will bind. Epitopes can comprise linear amino acid residues (i.e., residues within the epitope are arranged sequentially one after another in a linear fashion), nonlinear amino acid residues (referred to herein as "nonlinear epitopes"; these epitopes are not arranged sequentially), or both linear and nonlinear amino acid residues.

The term "CD40-antigen epitope" as used herein refers to a three dimensional molecular structure (either linear or conformational) that is capable of immunoreactivity with the anti-CD40 monoclonal antibodies of this invention, excluding the CD40 antigen itself. CD40-antigen epitopes may comprise proteins, protein fragments, peptides, carbohydrates, lipids, and other molecules, but for the purposes of the present invention are most commonly proteins, short oligopeptides, oligopeptide mimics (i.e., organic compounds which mimic the antibody binding properties of the CD40 antigen), or combinations thereof. Suitable oligopeptide mimics are described, *inter alia*, in PCT application US 91/04282.

Monoclonal antibodies can be prepared using the method of Kohler *et al.* (1975) *Nature* 256:495-496, or a modification thereof. Typically, a mouse is immunized with a solution containing an antigen. Immunization can be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally. Any



method of immunization known in the art may be used to obtain the monoclonal antibodies of the invention. After immunization of the animal, the spleen (and optionally, several large lymph nodes) are removed and dissociated into single cells. The spleen cells may be screened by applying a cell suspension to a plate or well coated with the antigen of interest. The B cells expressing membrane bound immunoglobulin specific for the antigen bind to the plate and are not rinsed away. Resulting B cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium. The resulting cells are plated by serial dilution and are assayed for the production of antibodies that specifically bind the antigen of interest (and that do not bind to unrelated antigens). The selected monoclonal antibody (mAb)-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

Where the antagonist anti-CD40 antibodies of the invention are to be prepared using recombinant DNA methods, the DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells described herein serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.* (1993) *Curr. Opinion in Immunol.* 5:256 and Phickthun (1992) *Immunol. Revs.* 130:151. As an alternative to the use of hybridomas, antibody can be produced in a cell line such as a CHO cell line, as disclosed in U.S. Patent Nos. 5,545,403; 5,545,405; and 5,998,144; incorporated herein by reference. Briefly the cell line is transfected with vectors capable of expressing a light chain and a heavy chain, respectively. By transfecting the two proteins on separate vectors, chimeric antibodies can be produced. Another advantage is the correct glycosylation of the antibody.

In some embodiments, the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 antibody, or antigen-binding fragment thereof is produced in CHO cells using the GS gene expression system (Lonza Biologics, Portsmouth, New Hampshire), which uses glutamine synthetase as a marker. See, also U.S. Patent Nos. 5,122,464; 5,591,639; 5,658,759; 5,770,359; 5,827,739; 5,879,936; 5,891,693; and 5,981,216; the contents of which are herein incorporated by reference in their entirety.

Monoclonal antibodies to CD40 are known in the art. See, for example, the sections dedicated to B-cell antigen in McMichael, ed. (1987; 1989) *Leukocyte Typing III and IV* (Oxford University Press, New York); U.S. Patent Nos. 5,674,492; 5,874,082; 5,677,165; 6,056,959; WO 00/63395; International Publication Nos. WO 02/28905 and WO 02/28904; Gordon *et al.* (1988) *J. Immunol.* 140:1425; Valle *et al.* (1989) *Eur. J. Immunol.* 19:1463; Clark *et al.* (1986) *PNAS* 83:4494; Paulie *et al.* (1989) *J. Immunol.* 142:590; Gordon *et al.* (1987) *Eur. J. Immunol.* 17:1535; Jabara *et al.* (1990) *J. Exp. Med.* 172:1861; Zhang *et al.* (1991) *J. Immunol.* 146:1836; Gascan *et al.* (1991) *J. Immunol.* 147:8; Banchereau *et al.* (1991) *Clin. Immunol. Spectrum* 3:8; and Banchereau *et al.* (1991) *Science* 251:70; all of which are herein incorporated by reference. Of particular interest to the present invention are the antagonist anti-CD40 antibodies disclosed herein that share the binding characteristics of the monoclonal antibodies CHIR-5.9 and CHIR-12.12 described above.

Additionally, the term "anti-CD40 antibody" as used herein encompasses chimeric anti-CD40 antibodies; such chimeric anti-CD40 antibodies for use in the methods of the invention have the binding characteristics of the CHIR-5.9 and CHIR-12.12 monoclonal antibodies described herein. By "chimeric" antibodies is intended antibodies that are most preferably derived using recombinant deoxyribonucleic acid techniques and which comprise both human (including immunologically "related" species, e.g., chimpanzee) and non-human components. Rituxan<sup>®</sup> is an example of a chimeric antibody with a murine variable region and a human constant region. For purposes of the present invention, the constant region of the chimeric antibody is most preferably substantially identical to the constant region of a natural human antibody; the variable region of the chimeric antibody is most preferably derived from a non-human source and has the desired antigenic specificity to the CD40 cell-surface antigen. The

non-human source can be any vertebrate source that can be used to generate antibodies to a human CD40 cell-surface antigen or material comprising a human CD40 cell-surface antigen. Such non-human sources include, but are not limited to, rodents (e.g., rabbit, rat, mouse, etc.; see, for example, U.S. Patent No. 4,816,567, herein incorporated by  
5 reference) and non-human primates (e.g., Old World Monkey, Ape, etc.; see, for example, U.S. Patent Nos. 5,750,105 and 5,756,096; herein incorporated by reference). As used herein, the phrase "immunologically active" when used in reference to chimeric anti-CD40 antibodies means a chimeric antibody that binds human CD40.

Humanized anti-CD40 antibodies represent additional anti-CD40 antibodies  
10 suitable for use in the methods of the present invention. By "humanized" is intended forms of anti-CD40 antibodies that contain minimal sequence derived from non-human immunoglobulin sequences. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (also known as complementarity determining region or CDR) of the recipient are  
15 replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and capacity. The phrase "complementarity determining region" refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. See, e.g., Chothia *et al* (1987) *J. Mol.*  
20 *Biol.* 196:901-917; Kabat *et al* (1991) U. S. Dept. of Health and Human Services, NIH Publication No. 91-3242). The phrase "constant region" refers to the portion of the antibody molecule that confers effector functions. In previous work directed towards producing non-immunogenic antibodies for use in therapy of human disease, mouse constant regions were substituted by human constant regions. The constant regions of the  
25 subject humanized antibodies were derived from human immunoglobulins. However, these humanized antibodies still elicited an unwanted and potentially dangerous immune response in humans and there was a loss of affinity. Humanized anti-CD40 antibodies for use in the methods of the present invention have binding characteristics similar to those exhibited by the CHIR-5.9 and CHIR-12.12 monoclonal antibodies described  
30 herein.

Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.* (1986) *Nature* 321:522-525; Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeven *et al.* (1988) *Science* 239:1534-1536), by substituting rodent or mutant rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. See also U.S. Patent Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; and 5,859,205; herein incorporated by reference. In some instances, residues within the framework regions of one or more variable regions of the human immunoglobulin are replaced by corresponding non-human residues (see, for example, U.S. Patent Nos. 5,585,089; 5,693,761; 5,693,762; and 6,180,370). Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance (e.g., to obtain desired affinity). In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones *et al.* (1986) *Nature* 331:522-525; Riechmann *et al.* (1988) *Nature* 332:323-329; and Presta (1992) *Curr. Op. Struct. Biol.* 2:593-596; herein incorporated by reference. Accordingly, such "humanized" antibodies may include antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies. See, for example, U.S. Patent Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205. See also U.S. Patent No. 6,180,370, and International Publication No. WO 01/27160, where humanized antibodies and techniques for producing humanized antibodies having improved affinity for a predetermined antigen are disclosed.

Also encompassed by the term anti-CD40 antibodies are xenogeneic or modified anti-CD40 antibodies produced in a non-human mammalian host, more particularly a transgenic mouse, characterized by inactivated endogenous immunoglobulin (Ig) loci. In

such transgenic animals, competent endogenous genes for the expression of light and heavy subunits of host immunoglobulins are rendered non-functional and substituted with the analogous human immunoglobulin loci. These transgenic animals produce human antibodies in the substantial absence of light or heavy host immunoglobulin subunits.

5 See, for example, U.S. Patent Nos. 5,877,397 and 5,939,598, herein incorporated by reference.

Preferably, fully human antibodies to CD40 are obtained by immunizing transgenic mice. One such mouse is obtained using XenoMouse<sup>®</sup> technology (Abgenix; Fremont, California) and is disclosed in U.S. Patent Nos. 6,075,181, 6,091,001, and  
10 6,114,598, all of which are incorporated herein by reference. To produce the antibodies disclosed herein, mice transgenic for the human Ig G<sub>1</sub> heavy chain locus and the human K light chain locus were immunized with Sf 9 cells expressing human CD40. Mice can also be transgenic for other isotypes. Fully human antibodies useful in the methods of the present invention are characterized by binding properties similar to those exhibited by the  
15 CHIR-5.9 and CHIR-12.12 monoclonal antibodies disclosed herein.

Fragments of the anti-CD40 antibodies are suitable for use in the methods of the invention so long as they retain the desired affinity of the full-length antibody. Thus, a fragment of an anti-CD40 antibody will retain the ability to bind to the CD40 B cell surface antigen. Such fragments are characterized by properties similar to the  
20 corresponding full-length antagonist anti-CD40 antibody, that is, the fragments will specifically bind a human CD40 antigen expressed on the surface of a human cell, and are free of significant agonist activity but exhibit antagonist activity when bound to a CD40 antigen on a human CD40-expressing cell. Such fragments are referred to herein as "antigen-binding" fragments.

25 Suitable antigen-binding fragments of an antibody comprise a portion of a full-length antibody, generally the antigen-binding or variable region thereof. Examples of antibody fragments include, but are not limited to, Fab, F(ab')<sub>2</sub>, and Fv fragments and single-chain antibody molecules. By "Fab" is intended a monovalent antigen-binding fragment of an immunoglobulin that is composed of the light chain and part of the heavy  
30 chain. By F(ab')<sub>2</sub> is intended a bivalent antigen-binding fragment of an immunoglobulin that contains both light chains and part of both heavy chains. By "single-chain Fv" or

"sFv" antibody fragments is intended fragments comprising the V<sub>H</sub> and V<sub>L</sub> domains of an antibody, wherein these domains are present in a single polypeptide chain. See, for example, U.S. Patent Nos. 4,946,778, 5,260,203, 5,455,030, and 5,856,456, herein incorporated by reference. Generally, the Fv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun (1994) in *The Pharmacology of Monoclonal Antibodies*, Vol. 113, ed. Rosenberg and Moore (Springer-Verlag, New York), pp. 269-315. Antigen-binding fragments of the antagonist anti-CD40 antibodies disclosed herein can also be conjugated to a cytotoxin to effect killing of the target cells, as described herein below.

Antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in, for example, McCafferty *et al.* (1990) *Nature* 348:552-554 (1990) and U.S. Patent No. 5,514,548. Clackson *et al.* (1991) *Nature* 352:624-628 and Marks *et al.* (1991) *J. Mol. Biol.* 222:581-597 describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.* (1992) *Bio/Technology* 10:779-783), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.* (1993) *Nucleic. Acids Res.* 21:2265-2266). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived *via* proteolytic digestion of intact antibodies (see, e.g., Morimoto *et al.* (1992) *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.* (1985) *Science* 229:81). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')<sub>2</sub> fragments (Carter *et al.* (1992) *Bio/Technology* 10:163-167). According to another approach, F(ab')<sub>2</sub> fragments can be isolated directly from

recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

Antagonist anti-CD40 antibodies useful in the methods of the present invention include the CHIR-5.9 and CHIR-12.12 monoclonal antibodies disclosed herein as well as  
5 antibodies differing from this antibody but retaining the CDRs; and antibodies with one or more amino acid addition(s), deletion(s), or substitution(s), wherein the antagonist activity is measured by inhibition of B-cell proliferation and/or differentiation. The invention also encompasses de-immunized antagonist anti-CD40 antibodies, which can be produced as described in, for example, International Publication Nos. WO 98/52976  
10 and WO 0034317; herein incorporated by reference. In this manner, residues within the antagonist anti-CD40 antibodies of the invention are modified so as to render the antibodies non- or less immunogenic to humans while retaining their antagonist activity toward human CD40-expressing cells, wherein such activity is measured by assays noted elsewhere herein. Also included within the scope of the claims are fusion proteins  
15 comprising an antagonist anti-CD40 antibody of the invention, or a fragment thereof, which fusion proteins can be synthesized or expressed from corresponding polynucleotide vectors, as is known in the art. Such fusion proteins are described with reference to conjugation of antibodies as noted below.

The antibodies of the present invention can have sequence variations produced  
20 using methods described in, for example, Patent Publication Nos. EP 0 983 303 A1, WO 00/34317, and WO 98/52976, incorporated herein by reference. For example, it has been shown that sequences within the CDR can cause an antibody to bind to MHC Class II and trigger an unwanted helper T cell response. A conservative substitution can allow the antibody to retain binding activity yet lose its ability to trigger an unwanted T cell  
25 response. Any such conservative or non-conservative substitutions can be made using art-recognized methods, such as those noted elsewhere herein, and the resulting antibodies will fall within the scope of the invention. The variant antibodies can be routinely tested for antagonist activity, affinity, and specificity using methods described herein.

30 The anti-CD40 antibodies useful in the practice of the invention can have one or many mechanisms of action. An antibody produced by any of the methods described

above, or any other method not disclosed herein, will fall within the scope of the invention if it possesses at least one of the following biological activities *in vitro* and/or *in vivo*: inhibition of immunoglobulin secretion by normal human peripheral B cells stimulated by T cells; inhibition of survival and/or proliferation of normal human peripheral B cells stimulated by CD40L-expressing cells or soluble CD40 ligand (sCD40L); inhibition of survival and/or proliferation of normal human peripheral B cells stimulated by Jurkat T cells; inhibition of "survival" anti-apoptotic intracellular signals in any cell stimulated by sCD40L or solid-phase CD40L; and, inhibition of CD40 signal transduction in any cell upon ligation with sCD40L or solid-phase CD40L, deletion, anergy and/or tolerance induction of CD40-bearing target cells or cells bearing cognate ligands to CD40 including, but not limited to, T cells and B cells, induction of expansion or activation of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (see for example, donor alloantigen-specific tissue rejection via CD40-CD40L interference, van Maurik *et al.* (2002) *J. Immunol.* 169:5401-5404), cytotoxicity via any mechanism (including, but not limited to, antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), down-regulation of proliferation, and/or apoptosis in target cells), modulation of target cell cytokine secretion and/or cell surface molecule expression, and combinations thereof. Assays for such biological activities can be performed as described herein and in copending provisional applications entitled "*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*," filed November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)), 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710 (Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety. See also the assays described in Schultze *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 92:8200-8204; Denton *et al.* (1998) *Pediatr. Transplant.* 2:6-15; Evans *et al.* (2000) *J. Immunol.* 164:688-697; Noelle (1998) *Agents Actions Suppl.* 49:17-22; Lederman *et al.* (1996) *Curr. Opin. Hematol.* 3:77-86; Coligan *et al.* (1991) *Current Protocols in Immunology* 13:12; Kwekkeboom *et al.* (1993) *Immunology* 79:439-444; and U.S. Patent Nos. 5,674,492 and 5,847,082; herein incorporated by reference.



A representative assay to detect antagonistic anti-CD40 antibodies specific to the CD40-antigen epitopes identified herein is a "competitive binding assay." Competitive binding assays are serological assays in which unknowns are detected and quantitated by their ability to inhibit the binding of a labeled known ligand to its specific antibody. This is also referred to as a competitive inhibition assay. In a representative competitive binding assay, labeled CD40 polypeptide is precipitated by candidate antibodies in a sample, for example, in combination with monoclonal antibodies raised against one or more epitopes of the monoclonal antibodies of the invention. Anti-CD40 antibodies that specifically react with an epitope of interest can be identified by screening a series of antibodies prepared against a CD40 protein or fragment of the protein comprising the particular epitope of the CD40 protein of interest. For example, for human CD40, epitopes of interest include epitopes comprising linear and/or nonlinear amino acid residues of the short isoform of human CD40 (see GenBank Accession No. NP\_690593) set forth in Figure 4B (SEQ ID NO:10), encoded by the sequence set forth in Figure 4A (SEQ ID NO:9; see also GenBank Accession No. NM\_152854), or of the long isoform of human CD40 (see GenBank Accession Nos. CAA43045 and NP\_001241) set forth in Figure 4D (SEQ ID NO:12), encoded by the sequence set forth in Figure 4C (SEQ ID NO:11; see GenBank Accession Nos. X60592 and NM\_001250). Alternatively, competitive binding assays with previously identified suitable antagonist anti-CD40 antibodies could be used to select monoclonal antibodies comparable to the previously identified antibodies.

Antibodies employed in such immunoassays may be labeled or unlabeled. Unlabeled antibodies may be employed in agglutination; labeled antibodies may be employed in a wide variety of assays, employing a wide variety of labels. Detection of the formation of an antibody-antigen complex between an anti-CD40 antibody and an epitope of interest can be facilitated by attaching a detectable substance to the antibody. Suitable detection means include the use of labels such as radionuclides, enzymes, coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, prosthetic group complexes, free radicals, particles, dyes, and the like. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes

include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material is luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$ , or  $^3\text{H}$ . Such labeled reagents may be used in a variety of well-known assays, such as radioimmunoassays, enzyme immunoassays, e.g., ELISA, fluorescent immunoassays, and the like. See for example, U.S. Patent Nos. 3,766,162; 3,791,932; 3,817,837; and 4,233,402.

Any of the previously described antagonist anti-CD40 antibodies or antibody fragments thereof may be conjugated prior to use in the methods of the present invention. Methods for producing conjugated antibodies are known in the art. Thus, the anti-CD40 antibody may be labeled using an indirect labeling or indirect labeling approach. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly  $^{32}\text{P}$  and  $^{125}\text{I}$ ), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefore. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example, HRP may serve as enzyme or as antigen for a mAb. Further, one may combine various labels for desired effect. For example, mAbs and avidin also require labels in the practice of this invention: thus, one might label a mAb with biotin, and detect its presence with avidin labeled with  $^{125}\text{I}$ , or with an anti-biotin mAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a therapeutic agent for example. The drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a protein such as CTLA4-Ig, an antibody or any other protein; or, biological response modifiers such as, for example, lymphokines, tumor necrosis factor, interferon-alpha, interferon-beta, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, BLyS (B Lymphocyte Stimulator), interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moieties to antibodies are well known. See, for example, Arnon *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld *et al.* (Alan R. Liss, Inc.), pp. 243-256; ed. Hellstrom *et al.* (1987), *Controlled Drug Delivery*, ed. Robinson *et al.* (2d ed; Marcel Dekker, Inc.), pp. 623-653; Thorpe (1985) *Monoclonal Antibodies '84: Biological and Clinical Applications*, ed. Pinchera *et al.* pp. 475-506 (Editrice Kurtis, Milano, Italy, 1985); *Monoclonal Antibodies for Cancer Detection and Therapy*, ed. Baldwin *et al.* (Academic Press, New York, 1985), pp. 303-316; and Thorpe *et al.* (1982) *Immunol. Rev.* 62:119-158.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described in U.S. Patent No. 4,676,980. In addition, linkers may be used between the labels and the antibodies of the invention (see U.S. Patent No. 4,831,175). Antibodies or, antigen-binding fragments thereof may be directly or indirectly labeled (U.S. Patent No. 5,595,721). Treatment may consist of a combination of treatment with conjugated and nonconjugated antibodies administered simultaneously or subsequently (WO 00/52031 and WO 00/52473).

#### Variants of Antagonist Anti-CD40 Antibodies

Suitable biologically active variants of the antagonist anti-CD40 antibodies can be used in the methods of the present invention. Such variants will retain the desired binding properties of the parent antagonist anti-CD40 antibody. Methods for making antibody variants are generally available in the art.

For example, amino acid sequence variants of an antagonist anti-CD40 antibody, for example, the CHIR-5.9 or CHIR-12.12 monoclonal antibody described herein, can be prepared by mutations in the cloned DNA sequence encoding the antibody of interest.

Methods for mutagenesis and nucleotide sequence alterations are well known in the art.

5 See, for example, Walker and Gaastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York); Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel *et al.* (1987) *Methods Enzymol.* 154:367-382; Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, New York); U.S. Patent No. 4,873,192; and the references cited therein; herein incorporated by reference.

10 Guidance as to appropriate amino acid substitutions that do not affect biological activity of the polypeptide of interest may be found in the model of Dayhoff *et al.* (1978) in *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred. Examples of  
15 conservative substitutions include, but are not limited to, Gly $\leftrightarrow$ Ala, Val $\leftrightarrow$ Ile $\leftrightarrow$ Leu, Asp $\leftrightarrow$ Glu, Lys $\leftrightarrow$ Arg, Asn $\leftrightarrow$ Gln, and Phe $\leftrightarrow$ Trp $\leftrightarrow$ Tyr.

In constructing variants of the antagonist anti-CD40 antibody polypeptide of interest, modifications are made such that variants continue to possess the desired activity, i.e., similar binding affinity and are capable of specifically binding to a human  
20 CD40 antigen expressed on the surface of a human cell, and being free of significant agonist activity but exhibiting antagonist activity when bound to a CD40 antigen on a human CD40-expressing cell. Obviously, any mutations made in the DNA encoding the variant polypeptide must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See  
25 EP Patent Application Publication No. 75,444.

In addition, the constant region of an antagonist anti-CD40 antibody can be mutated to alter effector function in a number of ways. For example, see U.S. Patent No. 6,737,056B1 and U.S. Patent Application Publication No. 2004/0132101A1, which disclose Fc mutations that optimize antibody binding to Fc receptors.

30 Preferably, variants of a reference antagonist anti-CD40 antibody have amino acid sequences that have at least 70% or 75% sequence identity, preferably at least 80% or

85% sequence identity, more preferably at least 90%, 91%, 92%, 93%, 94% or 95% sequence identity to the amino acid sequence for the reference antagonist anti-CD40 antibody molecule, for example, the CHIR-5.9 or CHIR-12.12 monoclonal antibody described herein, or to a shorter portion of the reference antibody molecule. More preferably, the molecules share at least 96%, 97%, 98% or 99% sequence identity. For purposes of the present invention, percent sequence identity is determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) *Adv. Appl. Math.* 2:482-489. A variant may, for example, differ from the reference antagonist anti-CD40 antibody by as few as 1 to 15 amino acid residues, as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

With respect to optimal alignment of two amino acid sequences, the contiguous segment of the variant amino acid sequence may have additional amino acid residues or deleted amino acid residues with respect to the reference amino acid sequence. The contiguous segment used for comparison to the reference amino acid sequence will include at least 20 contiguous amino acid residues, and may be 30, 40, 50, or more amino acid residues. Corrections for sequence identity associated with conservative residue substitutions or gaps can be made (see Smith-Waterman homology search algorithm).

The precise chemical structure of a polypeptide capable of specifically binding CD40 and retaining antagonist activity, particularly when bound to CD40 antigen on malignant B cells, depends on a number of factors. As ionizable amino and carboxyl groups are present in the molecule, a particular polypeptide may be obtained as an acidic or basic salt, or in neutral form. All such preparations that retain their biological activity when placed in suitable environmental conditions are included in the definition of antagonist anti-CD40 antibodies as used herein. Further, the primary amino acid sequence of the polypeptide may be augmented by derivatization using sugar moieties (glycosylation) or by other supplementary molecules such as lipids, phosphate, acetyl groups and the like. It may also be augmented by conjugation with saccharides. Certain aspects of such augmentation are accomplished through post-translational processing systems of the producing host; other such modifications may be introduced *in vitro*. In

any event, such modifications are included in the definition of an anti-CD40 antibody used herein so long as the antagonist properties of the anti-CD40 antibody are not destroyed. It is expected that such modifications may quantitatively or qualitatively affect the activity, either by enhancing or diminishing the activity of the polypeptide, in the various assays. Further, individual amino acid residues in the chain may be modified by oxidation, reduction, or other derivatization, and the polypeptide may be cleaved to obtain fragments that retain activity. Such alterations that do not destroy antagonist activity do not remove the polypeptide sequence from the definition of anti-CD40 antibodies of interest as used herein.

The art provides substantial guidance regarding the preparation and use of polypeptide variants. In preparing the anti-CD40 antibody variants, one of skill in the art can readily determine which modifications to the native protein nucleotide or amino acid sequence will result in a variant that is suitable for use as a therapeutically active component of a pharmaceutical composition used in the methods of the present invention.

#### Methods of Therapy Using the Antagonist Anti-CD40 Antibodies of the Invention

Methods of the invention are directed to the use of antagonist anti-CD40 antibodies to treat subjects (i.e., patients) having an autoimmune disease and/or inflammatory disease, or a predisposition to developing an autoimmune disease and/or inflammatory disease, wherein the disease and/or inflammation is mediated by CD40 ligand-mediated CD40 signaling on cells expressing the CD40 antigen. By the term "CD40-expressing cell," it is intended cells that express the CD40 antigen. Methods for detecting CD40 expression in cells are well known in the art and include, but are not limited to, PCR techniques, immunohistochemistry, flow cytometry, Western blot, ELISA, and the like.

The methods of the invention are especially useful for treating inflammatory and/or autoimmune diseases wherein CD40L-mediated CD40 stimulation is involved. The compositions of the invention may be administered prophylactically or therapeutically or a combination thereof.

Inflammatory diseases are characterized by inflammation and tissue destruction, or a combination thereof. "Inflammatory disease" includes any inflammatory immune-

mediated process where the initiating event or target of the immune response involves non-self antigen(s), including, for example, alloantigens, xenoantigens, viral antigens, bacterial antigens, unknown antigens, or allergens.

Further, for purposes of the present invention, the term "inflammatory disease(s)" includes "autoimmune disease(s)." As used herein, the term "autoimmunity" is generally understood to encompass inflammatory immune-mediated processes involving "self" antigens. In autoimmune diseases, self antigen(s) trigger host immune responses.

Also, the present invention includes treatment of inflammation associated with tissue transplant rejection. "Transplant rejection" or "graft rejection" refers to any host-mounted immune response against a graft including but not limited to HLA antigens, blood group antigens, and the like.

The invention can also be used to treat graft versus host disease, such as that associated with bone marrow transplantation, for example. In such graft versus host disease, the donor bone marrow includes lymphocytes and cells that mature into lymphocytes. The donor's lymphocytes recognize the recipient's antigens as non-self and mount an inflammatory immune response. Hence, as used herein, "graft versus host disease" or "graft versus host reaction" refers to any T cell mediated immune response in which donor lymphocytes react to the host's antigens.

The antagonist anti-CD40 antibodies and antigen-binding fragments thereof described herein can be used in accordance with the methods of the invention to treat autoimmune and/or inflammatory disorders including, but not limited to, systemic lupus erythematosus (SLE), discoid lupus, lupus nephritis, sarcoidosis, inflammatory arthritis, including juvenile arthritis, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, and gouty arthritis, rejection of an organ or tissue transplant, hyperacute, acute, or chronic rejection and/or graft versus host disease, multiple sclerosis, hyper IgE syndrome, polyarteritis nodosa, primary biliary cirrhosis, inflammatory bowel disease, Crohn's disease, celiac's disease (gluten-sensitive enteropathy), autoimmune hepatitis, pernicious anemia, autoimmune hemolytic anemia, psoriasis, scleroderma, myasthenia gravis, autoimmune thrombocytopenic purpura, autoimmune thyroiditis, Grave's disease, Hashimoto's thyroiditis, immune complex disease, chronic fatigue

immune dysfunction syndrome (CFIDS), polymyositis and dermatomyositis, cryoglobulinemia, thrombolysis, cardiomyopathy, pemphigus vulgaris, pulmonary interstitial fibrosis, Type I and Type II diabetes mellitus, type 1, 2, 3, and 4 delayed-type hypersensitivity, allergy or allergic disorders, unwanted/unintended immune responses to therapeutic proteins (see for example, U.S. Patent Application No. US 2002/0119151 and Koren, *et al.* (2002) *Curr. Pharm. Biotechnol.* 3:349-60), asthma, Churg-Strauss syndrome (allergic granulomatosis), atopic dermatitis, allergic and irritant contact dermatitis, urticaria, IgE-mediated allergy, atherosclerosis, vasculitis, idiopathic inflammatory myopathies, hemolytic disease, Alzheimer's disease, chronic inflammatory demyelinating polyneuropathy, and the like. In some other embodiments, the antagonistic anti-CD40 antibodies of the invention are useful in treating pulmonary inflammation including but not limited to lung graft rejection, asthma, sarcoidosis, emphysema, cystic fibrosis, idiopathic pulmonary fibrosis, chronic bronchitis, allergic rhinitis and allergic diseases of the lung such as hypersensitivity pneumonitis, eosinophilic pneumonia, bronchiolitis obliterans due to bone marrow and/or lung transplantation or other causes, graft atherosclerosis/graft phlebosclerosis, as well as pulmonary fibrosis resulting from collagen, vascular, and autoimmune diseases such as rheumatoid arthritis and lupus erythematosus.

"Treatment" is herein defined as the application or administration of an antagonist anti-CD40 antibody or antigen-binding fragment thereof to a subject, or application or administration of an antagonist anti-CD40 antibody or antigen-binding fragment thereof to an isolated tissue or cell line from a subject, where the subject has an autoimmune disease and/or inflammatory disease, a symptom associated with an autoimmune disease and/or inflammatory disease, or a predisposition toward development of an autoimmune disease and/or inflammatory disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the autoimmune disease and/or inflammatory disease, any associated symptoms of the autoimmune disease and/or inflammatory disease, or the predisposition toward the development of the autoimmune disease and/or inflammatory disease. By "treatment" is also intended the application or administration of a pharmaceutical composition comprising an antagonist anti-CD40 antibodies or antigen-binding fragment thereof to a subject, or application or



administration of a pharmaceutical composition comprising an antagonist anti-CD40 antibody or antigen-binding fragment thereof to an isolated tissue or cell line from a subject, where the subject has an autoimmune disease and/or inflammatory disease, a symptom associated with an autoimmune disease and/or inflammatory disease, or a predisposition toward development of an autoimmune disease and/or inflammatory disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the autoimmune disease and/or inflammatory disease, any associated symptoms of the autoimmune disease and/or inflammatory disease, or the predisposition toward the development of the autoimmune disease and/or inflammatory disease.

By "anti-inflammatory activity" is intended a reduction or prevention of inflammation. Therapy with at least one antagonist anti-CD40 antibody (or antigen-binding fragment thereof) as defined elsewhere herein causes a physiological response that is beneficial with respect to treatment of an autoimmune disease and/or inflammatory disease, where the disease involves cells expressing the CD40 antigen. It is recognized that the methods of the invention may be useful in preventing phenotypic change in cells such as proliferation, activation, and the like.

In accordance with the methods of the present invention, at least one antagonist anti-CD40 antibody (or antigen-binding fragment thereof) as defined elsewhere herein is used to promote a positive therapeutic response with respect to treatment or prevention of an autoimmune disease and/or inflammatory disease. By "positive therapeutic response" with respect to an autoimmune disease and/or inflammatory disease is intended an improvement in the disease in association with the anti-inflammatory activity of these antibodies or antigen-binding fragments thereof, and/or an improvement in the symptoms associated with the disease. That is, an anti-proliferative effect, the prevention of further proliferation of the CD40-expressing cell, a reduction in the inflammatory response including but not limited to reduced secretion of inflammatory cytokines, adhesion molecules, proteases, immunoglobulins (in instances where the CD40 bearing cell is a B cell), combinations thereof, and the like, increased production of anti-inflammatory proteins, a reduction in the number of autoreactive cells, an increase in immune tolerance, inhibition of autoreactive cell survival, and/or a decrease in one or more symptoms mediated by stimulation of CD40-expressing cells can be observed. Such positive

therapeutic responses are not limited to the route of administration and may comprise administration to the donor, the donor tissue (such as for example organ perfusion), the host, any combination thereof, and the like.

Clinical response can be assessed using screening techniques such as magnetic  
5 resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, flow cytometry or fluorescence-activated cell sorter (FACS) analysis, histology, gross pathology, and blood chemistry, including but not limited to changes detectable by ELISA, RIA, chromatography, and the like. In addition to these positive therapeutic responses, the subject undergoing therapy with the antagonist anti-CD40 antibody or  
10 antigen-binding fragment thereof may experience the beneficial effect of an improvement in the symptoms associated with the disease.

By "therapeutically effective dose or amount" or "effective amount" is intended an amount of antagonist anti-CD40 antibody or antigen-binding fragment thereof that, when administered brings about a positive therapeutic response with respect to treatment  
15 of a subject with an autoimmune disease and/or inflammatory disease. In some embodiments of the invention, a therapeutically effective dose of the anti-CD40 antibody or fragment thereof is in the range from about 0.01 mg/kg to about 40 mg/kg, from about 0.01 mg/kg to about 30 mg/kg, from about 0.1 mg/kg to about 30 mg/kg, from about 1 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 30 mg/kg, from about 3 mg/kg to  
20 about 25 mg/kg, from about 3 mg/kg to about 20 mg/kg, from about 5 mg/kg to about 15 mg/kg, or from about 7 mg/kg to about 12 mg/kg. It is recognized that the method of treatment may comprise a single administration of a therapeutically effective dose or multiple administrations of a therapeutically effective dose of the antagonist anti-CD40 antibody or antigen-binding fragment thereof.

25 A further embodiment of the invention is the use of antagonist anti-CD40 antibodies for diagnostic monitoring of protein levels in tissue as part of a clinical testing procedure, e.g., to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials,  
30 luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase,

or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material  
5 includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$ , or  $^3\text{H}$ .

The antagonist anti-CD40 antibodies and suitable antigen-binding fragments thereof can be used in combination with any known therapies for autoimmune and inflammatory diseases, including any agent or combination of agents that are known to be  
10 useful, or which have been used or are currently in use, for treatment of autoimmune and inflammatory diseases. Such therapies and therapeutic agents include, but are not limited to, surgery or surgical procedures (e.g. splenectomy, lymphadenectomy, thyroidectomy, plasmaphoresis, leukapheresis, cell, tissue, or organ transplantation, intestinal procedures, organ perfusion, and the like), radiation therapy, therapy such as steroid  
15 therapy and non-steroidal therapy, hormone therapy, cytokine therapy, therapy with dermatological agents (for example, topical agents used to treat skin conditions such as allergies, contact dermatitis, and psoriasis), immunosuppressive therapy, and other anti-inflammatory monoclonal antibody therapy, and the like. In this manner, the antagonist anti-CD40 antibodies described herein, or antigen-binding fragments thereof, are  
20 administered in combination with at least one other therapy, including, but not limited to, surgery, organ perfusion, radiation therapy, steroid therapy, non-steroidal therapy, antibiotic therapy, antifungal therapy, hormone therapy, cytokine therapy, therapy with dermatological agents (for example, topical agents used to treat skin conditions such as allergies, contact dermatitis, and psoriasis), immunosuppressive therapy, other anti-  
25 inflammatory monoclonal antibody therapy, combinations thereof, and the like. Thus, where the combined therapies comprise administration of an antagonist anti-CD40 antibody or antigen-binding fragment thereof in combination with administration of another therapeutic agent, as with steroids as one example, the methods of the invention encompass coadministration, using separate formulations or a single pharmaceutical  
30 formulation, and consecutive administration in either order.

Where the methods of the present invention comprise combined therapeutic regimens, these therapies can be given simultaneously, i.e., the antagonist anti-CD40 antibody or antigen-binding fragment thereof is administered concurrently or within the same time frame as the other therapy (i.e., the therapies are going on concurrently, but the anti-CD40 antibody or antigen-binding fragment thereof is not administered precisely at the same time as the other therapy). Alternatively, the antagonist anti-CD40 antibody of the present invention or antigen-binding fragment thereof may also be administered prior to or subsequent to the other therapy. Sequential administration of the different therapies may be performed regardless of whether the treated subject responds to the first course of therapy to decrease the possibility of remission or relapse.

In some embodiments of the invention, the antagonist anti-CD40 antibodies described herein, or antigen-binding fragments thereof, are administered in combination with immunosuppressive drugs or anti-inflammatory drugs, wherein the antibody and the therapeutic agent(s) may be administered sequentially, in either order, or simultaneously (i.e., concurrently or within the same time frame). Examples of suitable immunosuppressive drugs that can be administered in combination with the antagonistic anti-CD40 antibodies of the invention include, but are not limited to, methotrexate, cyclophosphamide, mizoribine, chlorambucil, cyclosporine, such as, for example, aerosolized cyclosporine (see, U.S. Patent Application Publication No. US20020006901, herein incorporated by reference in its entirety), tacrolimus (FK506; ProGraf™), mycophenolate mofetil, and azathioprine (6-mercaptopurine), sirolimus (rapamycin), deoxyspergualin, leflunomide and its malononitriloamide analogs; and immunosuppressive proteins, including, for example, anti-CTLA4 antibodies and Ig fusions, anti-B lymphocyte stimulator antibodies (e.g., LYMPHOSTAT-B™) and Ig fusions (BLyS-Ig), anti-CD80 antibodies and etanercept (Enbrel®), as well as anti-T cell antibodies such as anti-CD3 (OKT3), anti-CD4, and the like. Examples of suitable anti-inflammatory agents include, but are not limited to, corticosteroids such as, for example, clobetasol, halobetasol, hydrocortisone, triamcinolone, betamethasone, fluocinole, fluocinonide, prednisone, prednisolone, methylprednisolone; non-steroidal anti-inflammatory drugs (NSAIDs) such as, for example, sulfasalazine, medications containing mesalamine (known as 5-ASA agents), celecoxib, diclofenac, etodolac,

fenprofen, flurbiprofen, ibuprofen, ketoprofen, meclofamate, meloxicam, nabumetone, naproxen, oxaprozin, piroxicam, rofecoxib, salicylates, sulindac, and tolmetin; anti-inflammatory antibodies such as adalimumab (HUMIRA<sup>®</sup>, a TNF- $\alpha$  antagonist) and infliximab (Remicade<sup>®</sup>, a TNF- $\alpha$  antagonist), and the like.

5 Transplant rejection and graft versus host disease can be hyperacute (humoral), acute (T cell mediated), or chronic (unknown etiology), or a combination thereof. Thus, the antagonistic anti-CD40 antibodies of the invention are used in some embodiments to prevent and/or ameliorate rejection and/or symptoms associated with hyperacute, acute, and/or chronic transplant rejection of any tissue, including, but not limited to, liver,  
10 kidney, pancreas, pancreatic islet cells, small intestine, lung, heart, corneas, skin, blood vessels, bone, heterologous or autologous bone marrow, and the like. Graft tissues may be obtained from any donor and transplanted into any recipient host, and thus the transplant procedure may comprise transplanting animal tissue to humans (e.g., xenografts), transplanting tissue from one human to another human (e.g., allografts),  
15 and/or transplanting tissue from one part of a human's body to another (e.g., autografts). Treatment with the antibodies of the invention may also reduce transplantation sequelae such as fever, anorexia, hemodynamic abnormalities, leukopenia, white cell infiltration of the transplanted organ/tissue, as well as opportunistic infections.

In some embodiments, the antagonistic anti-CD40 antibodies of the invention  
20 may be used alone or in combination with immunosuppressive drugs to treat and/or prevent transplant rejection such as hyperacute, acute, and/or chronic rejection and/or graft versus host disease. Thus, in some embodiments where the antagonistic anti-CD40 antibodies of the invention are used to treat graft rejection, the antibodies may be used in combination with suitable immunosuppressive drugs, including, but not limited, to  
25 methotrexate; cyclophosphamide; mizoribine; chlorambucil; cyclosporine, such as, for example, aerosolized cyclosporine (see, U.S. Patent Application Publication No. US20020006901, herein incorporated by reference in its entirety), tacrolimus (FK506; ProGraft<sup>™</sup>), mycophenolate mofetil, and azathioprine (6-mercaptopurine), sirolimus (rapamycin), deoxyspergualin, leflunomide and its malononitriloamide analogs; and  
30 immunosuppressive proteins, including, for example, anti-CTLA antibodies and Ig fusions, anti-B lymphocyte stimulator antibodies (e.g., LYMPHOSTAT-B<sup>™</sup>) and Ig

fusions (BLyS-Ig), anti-CD80 antibodies and etanercept (Enbrel<sup>®</sup>), as well as anti-T cell antibodies such as anti-CD3 (OKT3), anti-CD4, and the like.

As such, it is specifically contemplated that the compositions and methods of the invention are used in combination with other drugs to further improve symptoms and outcomes in transplant recipients, such as those receiving lung grafts, for example. Thus, in some embodiments, the antagonistic anti-CD40 antibodies of the invention are used to treat transplant rejection (such as, for example hyperacute, acute, and/or chronic rejection or graft versus host disease in lung transplant recipients) alone or in combination with parenterally and/or non-parenterally administered cyclosporine, including for example oral cyclosporine, injectable cyclosporine, aerosolized (e.g., inhaled) cyclosporine, and combinations thereof. In some embodiments where at least a component of the therapy is aerosolized cyclosporine, the cyclosporine is delivered to the lung of the recipient by inhalation of cyclosporine in aerosol spray form using, for example, a pressurized delivery device or nebulizer. The cyclosporine may be administered in either dry powder or wet form.

In some other embodiments, the antagonistic anti-CD40 antibodies of the invention may be used alone or in combination with immunosuppressive drugs to treat and/or prevent rheumatoid arthritis. Thus in some embodiments where the antagonistic anti-CD40 antibodies of the invention are used to treat rheumatoid arthritis, the antibodies may be used in combination with suitable immunosuppressive drugs, including, but not limited to, methotrexate, cyclophosphamide, mizoribine, chlorambucil, cyclosporine, tacrolimus (FK506; PROGRAF<sup>™</sup>), mycophenolate mofetil, and azathioprine (6-mercaptopurine), sirolimus (rapamycin), deoxyspergualin, leflunomide and its malononitriloamide analogs; and immunosuppressive proteins, including, for example, anti-CTLA antibodies and Ig fusions, anti-B lymphocyte stimulator antibodies (e.g., LYMPHOSTAT-B<sup>™</sup>) and Ig fusions (BLyS-Ig), anti-CD20 antibodies (e.g. RITUXAN<sup>®</sup>); the fully human antibody HuMax-CD20, R-1594, IMMU-106, TRU-015, AME-133, tositumomab/I-131, tositumomab (Bexxar<sup>®</sup>), ibritumomab tituxetan (Zevalin<sup>®</sup>); anti-CD80 antibodies, and etanercept (ENBREL<sup>®</sup>), as well as anti-T cell antibodies such as anti-CD3 (OKT3), anti-CD4, and the like. As discussed above, treatment effectiveness may be assessed using any means and includes, but is not limited

to, effectiveness as measured by clinical responses defined by the American College of Rheumatology criteria, the European League of Rheumatism criteria, or any other criteria. See for example, Felson *et al.* (1995) *Arthritis. Rheum.* 38:727-35 and van Gestel *et al.* (1996) *Arthritis Rheum.* 39:34-40.

5           In yet other embodiments, the antagonistic anti-CD40 antibodies of the invention may be used alone or in combination with immunosuppressive drugs to treat and/or prevent multiple sclerosis. Thus in some embodiments where the antagonistic anti-CD40 antibodies of the invention are used to treat multiple sclerosis, the antibodies may be used in combination with suitable immunosuppressive drugs, including, but not limited to,  
10   methotrexate, cyclophosphamide, mizoribine, chlorambucil, cyclosporine, tacrolimus (FK506; PROGRAF<sup>TM</sup>), mycophenolate mofetil, and azathioprine (6-mercaptopurine), sirolimus (rapamycin), deoxyspergualin, leflunomide and its malononitriloamide analogs; and immunosuppressive proteins, including, for example, anti-CTLA antibodies and Ig fusions, anti-B lymphocyte stimulator antibodies (e.g., LYMPHOSTAT-B<sup>TM</sup>) and Ig  
15   fusions (BLyS-Ig), anti-CD20 antibodies (e.g., RITUXAN<sup>®</sup>); the fully human antibody HuMax-CD20, R-1594, IMMU-106, TRU-015, AME-133, tositumomab/I-131, tositumomab (Bexxar<sup>®</sup>), ibritumomab tituxetan (Zevalin<sup>®</sup>); anti-CD80 antibodies, and etanercept (ENBREL<sup>®</sup>), as well as anti-T cell antibodies such as anti-CD3 (OKT3), anti-CD4, and the like.

20

#### Pharmaceutical Formulations and Modes of Administration

The antagonist anti-CD40 antibodies of this invention are administered at a concentration that is therapeutically effective to prevent or treat autoimmune diseases and/or inflammatory diseases. To accomplish this goal, the antibodies may be formulated  
25   using a variety of acceptable excipients known in the art. Typically, the antibodies are administered by injection, for example, either intravenously, intraperitoneally, or subcutaneously. Methods to accomplish this administration are known to those of ordinary skill in the art. It may also be possible to obtain compositions that may be topically or orally administered, or which may be capable of transmission across mucous  
30   membranes.

Intravenous administration occurs preferably by infusion over a period of about 1 to about 10 hours, more preferably over about 1 to about 8 hours, even more preferably over about 2 to about 7 hours, still more preferably over about 4 to about 6 hours, depending upon the anti-CD40 antibody being administered. The initial infusion with the pharmaceutical composition may be given over a period of about 4 to about 6 hours with subsequent infusions delivered more quickly. Subsequent infusions may be administered over a period of about 1 to about 6 hours, including, for example, about 1 to about 4 hours, about 1 to about 3 hours, or about 1 to about 2 hours.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of possible routes of administration include parenteral, (e.g., intravenous (IV), intramuscular (IM), intradermal, subcutaneous (SC), or infusion), oral and pulmonary (e.g., inhalation), nasal, transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic.

The antagonist anti-CD40 antibodies are typically provided by standard technique within a pharmaceutically acceptable buffer, for example, sterile saline, sterile buffered water, propylene glycol, combinations of the foregoing, etc. Methods for preparing parenterally administrable agents are described in *Remington's Pharmaceutical Sciences* (18<sup>th</sup> ed.; Mack Publishing Company, Eaton, Pennsylvania, 1990), herein incorporated by reference. See also, for example, WO 98/56418, which describes stabilized antibody pharmaceutical formulations suitable for use in the methods of the present invention.

The amount of at least one antagonist anti-CD40 antibody or fragment thereof to be administered is readily determined by one of ordinary skill in the art without undue



experimentation. Factors influencing the mode of administration and the respective amount of at least one antagonist anti-CD40 antibody (or fragment thereof) include, but are not limited to, the particular disease undergoing therapy, the severity of the disease, the history of the disease, and the age, height, weight, health, and physical condition of the individual undergoing therapy. Similarly, the amount of antagonist anti-CD40 antibody or fragment thereof to be administered will be dependent upon the mode of administration and whether the subject will undergo a single dose or multiple doses of this agent. Generally, a higher dosage of anti-CD40 antibody or fragment thereof is preferred with increasing weight of the patient undergoing therapy. The dose of anti-CD40 antibody or fragment thereof to be administered is in the range from about 0.003 mg/kg to about 50 mg/kg, preferably in the range of 0.01 mg/kg to about 40 mg/kg. Thus, for example, the dose can be 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 5 mg/kg, 7 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, or 50 mg/kg.

In another embodiment of the invention, the method comprises administration of multiple doses of antagonist anti-CD40 antibody or fragment thereof. The method may comprise administration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, or more therapeutically effective doses of a pharmaceutical composition comprising an antagonist anti-CD40 antibody or fragment thereof. The frequency and duration of administration of multiple doses of the pharmaceutical compositions comprising anti-CD40 antibody or fragment thereof can be readily determined by one of skill in the art without undue experimentation. Moreover, treatment of a subject with a therapeutically effective amount of an antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antagonist anti-CD40 antibody or antigen-binding fragment thereof in the range of between about 0.1 to 20 mg/kg body weight, once per week for between about 1 to 10 weeks, preferably between about 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. Treatment may occur annually to prevent relapse or upon indication of relapse. It will also be appreciated that the effective dosage of antibody or antigen-binding fragment thereof used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become

apparent from the results of diagnostic assays as described herein. Thus, in one embodiment, the dosing regimen includes a first administration of a therapeutically effective dose of at least one anti-CD40 antibody or fragment thereof on days 1, 7, 14, and 21 of a treatment period. In another embodiment, the dosing regimen includes a first administration of a therapeutically effective dose of at least one anti-CD40 antibody or fragment thereof on days 1, 2, 3, 4, 5, 6, and 7 of a week in a treatment period. Further embodiments include a dosing regimen having a first administration of a therapeutically effective dose of at least one anti-CD40 antibody or fragment thereof on days 1, 3, 5, and 7 of a week in a treatment period; a dosing regimen including a first administration of a therapeutically effective dose of at least one anti-CD40 antibody or fragment thereof on days 1 and 3 of a week in a treatment period; and a preferred dosing regimen including a first administration of a therapeutically effective dose of at least one anti-CD40 antibody or fragment thereof on day 1 of a week in a treatment period. The treatment period may comprise 1 week, 2 weeks, 3 weeks, a month, 3 months, 6 months, or a year. Treatment periods may be subsequent or separated from each other by a day, a week, 2 weeks, a month, 3 months, 6 months, or a year.

ranges from about 0.003 mg/kg to about 50 mg/kg, from about 0.01 mg/kg to about 40 mg/kg, from about 0.01 mg/kg to about 30 mg/kg, from about 0.1 mg/kg to about 30 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 1 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 25 mg/kg, from about 3 mg/kg to about 20 mg/kg, from about 5 mg/kg to about 15 mg/kg, or from about 7 mg/kg to about 12 mg/kg. Thus, for example, the dose of any one antagonist anti-CD40 antibody or antigen-binding fragment thereof, for example the anti-CD40 monoclonal antibody CHIR-12.12 or CHIR-5.9 or antigen-binding fragment thereof, can be 0.003 mg/kg, 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 5 mg/kg, 7 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, or other such doses falling within the range of about 0.003 mg/kg to about 50 mg/kg. The same therapeutically effective dose of an antagonist anti-CD40 antibody or antigen-binding fragment thereof can be administered throughout each week of antibody dosing. Alternatively, different therapeutically effective doses of an antagonist anti-CD40

antibody or antigen-binding fragment thereof can be used over the course of a treatment period.

In some embodiments, the initial therapeutically effective dose of an antagonist anti-CD40 antibody or antigen-binding fragment thereof as defined elsewhere herein can be in the lower dosing range (i.e., about 0.003 mg/kg to about 20 mg/kg) with subsequent doses falling within the higher dosing range (i.e., from about 20 mg/kg to about 50 mg/kg).

In alternative embodiments, the initial therapeutically effective dose of an antagonist anti-CD40 antibody or antigen-binding fragment thereof as defined elsewhere herein can be in the upper dosing range (i.e., about 20 mg/kg to about 50 mg/kg) with subsequent doses falling within the lower dosing range (i.e., 0.003 mg/kg to about 20 mg/kg). Thus, in one embodiment, the initial therapeutically effective dose of the antagonist anti-CD40 antibody or antigen-binding fragment thereof is about 20 mg/kg to about 35 mg/kg, including about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, and about 35 mg/kg, and subsequent therapeutically effective doses of the antagonist anti-CD40 antibody or antigen binding fragment thereof are about 5 mg/kg to about 15 mg/kg, including about 5 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, and about 15 mg/kg.

In some embodiments of the invention, antagonist anti-CD40 antibody therapy is initiated by administering a "loading dose" of the antibody or antigen-binding fragment thereof to the subject in need of antagonist anti-CD40 antibody therapy. By "loading dose" is intended an initial dose of the antagonist anti-CD40 antibody or antigen-binding fragment thereof that is administered to the subject, where the dose of the antibody or antigen-binding fragment thereof administered falls within the higher dosing range (i.e., from about 20 mg/kg to about 50 mg/kg). The "loading dose" can be administered as a single administration, for example, a single infusion where the antibody or antigen-binding fragment thereof is administered IV, or as multiple administrations, for example, multiple infusions where the antibody or antigen-binding fragment thereof is administered IV, so long as the complete "loading dose" is administered within about a 24-hour period. Following administration of the "loading dose," the subject is then administered one or more additional therapeutically effective doses of the antagonist anti-CD40 antibody or antigen-binding fragment thereof. Subsequent therapeutically

effective doses can be administered, for example, according to a weekly dosing schedule, or once every two weeks, once every three weeks, or once every four weeks. In such embodiments, the subsequent therapeutically effective doses generally fall within the lower dosing range (i.e., 0.003 mg/kg to about 20 mg/kg).

5           Alternatively, in some embodiments, following the "loading dose," the subsequent therapeutically effective doses of the antagonist anti-CD40 antibody or antigen-binding fragment thereof are administered according to a "maintenance schedule," wherein the therapeutically effective dose of the antibody or antigen-binding  
10           fragment thereof is administered once a month, once every 6 weeks, once every two months, once every 10 weeks, once every three months, once every 14 weeks, once every four months, once every 18 weeks, once every five months, once every 22 weeks, once  
15           every six months, once every 7 months, once every 8 months, once every 9 months, once every 10 months, once every 11 months, or once every 12 months. In such embodiments, the therapeutically effective doses of the antagonist anti-CD40 antibody or antigen-  
20           binding fragment thereof fall within the lower dosing range (i.e., 0.003 mg/kg to about 20 mg/kg), particularly when the subsequent doses are administered at more frequent intervals, for example, once every two weeks to once every month, or within the higher dosing range (i.e., from about 20 mg/kg to about 50 mg/kg), particularly when the subsequent doses are administered at less frequent intervals, for example, where  
25           subsequent doses are administered about one month to about 12 months apart.

          The antagonist anti-CD40 antibodies present in the pharmaceutical compositions described herein for use in the methods of the invention may be native or obtained by recombinant techniques, and may be from any source, including mammalian sources such  
30           as, e.g., mouse, rat, rabbit, primate, pig, and human. Preferably such polypeptides are derived from a human source, and more preferably are recombinant, human proteins from hybridoma cell lines.

          The pharmaceutical compositions useful in the methods of the invention may comprise biologically active variants of the antagonist anti-CD40 antibodies of the invention. Such variants should retain the desired biological activity of the native  
35           polypeptide such that the pharmaceutical composition comprising the variant polypeptide has the same therapeutic effect as the pharmaceutical composition comprising the native

polypeptide when administered to a subject. That is, the variant anti-CD40 antibody will serve as a therapeutically active component in the pharmaceutical composition in a manner similar to that observed for the native antagonist antibody, for example CHIR-5.9 or CHIR-12.12 as expressed by the hybridoma cell line 5.9 or 12.12, respectively.

5 Methods are available in the art for determining whether a variant anti-CD40 antibody retains the desired biological activity, and hence serves as a therapeutically active component in the pharmaceutical composition. Biological activity of antibody variants can be measured using assays specifically designed for measuring activity of the native antagonist antibody, including assays described in the present invention.

10 Any pharmaceutical composition comprising an antagonist anti-CD40 antibody having the binding properties described herein as the therapeutically active component can be used in the methods of the invention. Thus liquid, lyophilized, or spray-dried compositions comprising one or more of the antagonist anti-CD40 antibodies of the invention may be prepared as an aqueous or nonaqueous solution or suspension for  
15 subsequent administration to a subject in accordance with the methods of the invention. Each of these compositions will comprise at least one of the antagonist anti-CD40 antibodies of the present invention as a therapeutically or prophylactically active component. By "therapeutically or prophylactically active component" is intended the anti-CD40 antibody is specifically incorporated into the composition to bring about a  
20 desired therapeutic or prophylactic response with regard to treatment, prevention, or diagnosis of a disease or condition within a subject when the pharmaceutical composition is administered to that subject. Preferably the pharmaceutical compositions comprise appropriate stabilizing agents, bulking agents, or both to minimize problems associated with loss of protein stability and biological activity during preparation and storage.

25 Formulants may be added to pharmaceutical compositions comprising an antagonist anti-CD40 antibody of the invention. These formulants may include, but are not limited to, oils, polymers, vitamins, carbohydrates, amine acids, salts, buffers, albumin, surfactants, or bulking agents. Preferably carbohydrates include sugar or sugar alcohols such as mono-, di-, or polysaccharides, or water soluble glucans. The  
30 saccharides or glucans can include fructose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin,  $\alpha$  and  $\beta$  cyclodextrin, soluble starch, hydroxyethyl

starch, and carboxymethylcellulose, or mixtures thereof. "Sugar alcohol" is defined as a C<sub>4</sub> to C<sub>8</sub> hydrocarbon having a hydroxyl group and includes galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. These sugars or sugar alcohols may be used individually or in combination. The sugar or sugar alcohol concentration is between 1.0% and 7% w/v., more preferably between 2.0% and 6.0% w/v. Preferably amino acids include levorotary (L) forms of carnitine, arginine, and betaine; however, other amino acids may be added. Preferred polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000. Surfactants that can be added to the formulation are shown in EP Nos. 270,799 and 268,110.

Additionally, antibodies can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Preferred polymers, and methods to attach them to peptides, are shown in U.S. Patent Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546; which are all hereby incorporated by reference in their entireties. Preferred polymers are polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: R(O--CH<sub>2</sub>--CH<sub>2</sub>)<sub>n</sub> O--R where R can be hydrogen, or a protective group such as an alkyl or alkanol group. Preferably, the protective group has between 1 and 8 carbons, more preferably it is methyl. The symbol n is a positive integer, preferably between 1 and 1,000, more preferably between 2 and 500. The PEG has a preferred average molecular weight between 1,000 and 40,000, more preferably between 2,000 and 20,000, most preferably between 3,000 and 12,000. Preferably, PEG has at least one hydroxy group, more preferably it is a terminal hydroxy group. It is this hydroxy group which is preferably activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/antibody of the present invention.

Water-soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), and the like. POG is preferred. One reason is because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, triglycerides. Therefore, this branching

would not necessarily be seen as a foreign agent in the body. The POG has a preferred molecular weight in the same range as PEG. The structure for POG is shown in Knauf *et al.* (1988) *J. Bio. Chem.* 263:15064-15070, and a discussion of POG/IL-2 conjugates is found in U.S. Patent No. 4,766,106, both of which are hereby incorporated by reference in  
5 their entireties.

Another drug delivery system for increasing circulatory half-life is the liposome. Methods of preparing liposome delivery systems are discussed in Gabizon *et al.* (1982) *Cancer Research* 42:4734; Cafiso (1981) *Biochem Biophys Acta* 649:129; and Szoka (1980) *Ann. Rev. Biophys. Eng.* 9:467. Other drug delivery systems are known in the art  
10 and are described in, e.g., Poznansky *et al.* (1980) *Drug Delivery Systems* (R.L. Juliano, ed., Oxford, N.Y.) pp. 253-315; Poznansky (1984) *Pharm Revs* 36:277.

The formulants to be incorporated into a pharmaceutical composition should provide for the stability of the antagonist anti-CD40 antibody or antigen-binding fragment thereof. That is, the antagonist anti-CD40 antibody or antigen-binding fragment  
15 thereof should retain its physical and/or chemical stability and have the desired biological activity, i.e., one or more of the antagonist activities defined herein above, including, but not limited to, inhibition of immunoglobulin secretion by normal human peripheral B cells stimulated by T cells; inhibition of survival and/or proliferation of normal human peripheral B cells stimulated by Jurkat T cells; inhibition of survival and/or proliferation  
20 of normal human peripheral B cells stimulated by CD40L-expressing cells or soluble CD40 ligand (sCD40L); inhibition of "survival" anti-apoptotic intracellular signals in any cell stimulated by sCD40L or solid-phase CD40L; inhibition of CD40 signal transduction in any cell upon ligation with sCD40L or solid-phase CD40L; and inhibition of proliferation of human malignant B cells as noted elsewhere herein.

25 Methods for monitoring protein stability are well known in the art. See, for example, Jones (1993) *Adv. Drug Delivery Rev.* 10:29-90; Lee, ed. (1991) *Peptide and Protein Drug Delivery* (Marcel Dekker, Inc., New York, New York); and the stability assays disclosed herein below. Generally, protein stability is measured at a chosen temperature for a specified period of time. In preferred embodiments, a stable antibody  
30 pharmaceutical formulation provides for stability of the antagonist anti-CD40 antibody or antigen-binding fragment thereof when stored at room temperature (about 25°C) for at

least 1 month, at least 3 months, or at least 6 months, and/or is stable at about 2-8°C for at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months.

5 A protein such as an antibody, when formulated in a pharmaceutical composition, is considered to retain its physical stability at a given point in time if it shows no visual signs (i.e., discoloration or loss of clarity) or measurable signs (for example, using size-exclusion chromatography (SEC) or UV light scattering) of precipitation, aggregation, and/or denaturation in that pharmaceutical composition. With respect to chemical stability, a protein such as an antibody, when formulated in a pharmaceutical  
10 composition, is considered to retain its chemical stability at a given point in time if measurements of chemical stability are indicative that the protein (i.e., antibody) retains the biological activity of interest in that pharmaceutical composition. Methods for monitoring changes in chemical stability are well known in the art and include, but are not limited to, methods to detect chemically altered forms of the protein such as result  
15 from clipping, using, for example, SDS-PAGE, SEC, and/or matrix-assisted laser desorption ionization/time of flight mass spectrometry; and degradation associated with changes in molecular charge (for example, associated with deamidation), using, for example, ion-exchange chromatography. See, for example, the methods disclosed herein below.

20 An antagonist anti-CD40 antibody or antigen-binding fragment thereof, when formulated in a pharmaceutical composition, is considered to retain a desired biological activity at a given point in time if the desired biological activity at that time is within about 30%, preferably within about 20% of the desired biological activity exhibited at the time the pharmaceutical composition was prepared as determined in a suitable assay for  
25 the desired biological activity. Assays for measuring the desired biological activity of the antagonist anti-CD40 antibodies disclosed herein, and antigen-binding fragments thereof, can be performed as described in the Examples herein. See also the assays described in Schultze *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 92:8200-8204; Denton *et al.* (1998) *Pediatr. Transplant.* 2:6-15; Evans *et al.* (2000) *J. Immunol.* 164:688-697; Noelle (1998)  
30 *Agents Actions Suppl.* 49:17-22; Lederman *et al.* (1996) *Curr. Opin. Hematol.* 3:77-86; Coligan *et al.* (1991) *Current Protocols in Immunology* 13:12; Kwekkeboom *et al.* (1993)



*Immunology* 79:439-444; and U.S. Patent Nos. 5,674,492 and 5,847,082; herein incorporated by reference.

In some embodiments of the invention, the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding  
5 fragment thereof is formulated in a liquid pharmaceutical formulation. The antagonist anti-CD40 antibody or antigen binding fragment thereof can be prepared using any method known in the art, including those methods disclosed herein above. In one embodiment, the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-  
10 5.9 monoclonal antibody, or antigen-binding fragment thereof is recombinantly produced in a CHO cell line.

Following its preparation and purification, the antagonist anti-CD40 antibody or antigen-binding fragment thereof can be formulated as a liquid pharmaceutical formulation in the manner set forth herein. Where the antagonist anti-CD40 antibody or antigen-binding fragment thereof is to be stored prior to its formulation, it can be frozen,  
15 ro example, at  $\leq -20^{\circ}\text{C}$ , and then thawed at room temperature for further formulation. The liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody or antigen-binding fragment thereof. The amount of antibody or antigen-binding fragment thereof present in the formulation takes into consideration the route of administration and desired dose volume.

In this manner, the liquid pharmaceutical composition comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 antibody, or antigen-binding fragment thereof at a concentration of about 0.1 mg/ml to about 50.0 mg/ml, about 0.5 mg/ml to about 40.0 mg/ml, about 1.0 mg/ml to about 30.0 mg/ml, about 5.0 mg/ml to about 25.0 mg/ml, about 5.0 mg/ml to about 20.0 mg/ml, or about 15.0 mg/ml  
25 to about 25.0 mg/ml. In some embodiments, the liquid pharmaceutical composition comprises the antagonist anti-CD40 antibody or antigen-binding fragment thereof at a concentration of about 0.1 mg/ml to about 5.0 mg/ml, about 5.0 mg/ml to about 10.0 mg/ml, about 10.0 mg/ml to about 15.0 mg/ml, about 15.0 mg/ml to about 20.0 mg/ml, about 20.0 mg/ml to about 25.0 mg/ml, about 25.0 mg/ml to about 30.0 mg/ml, about  
30 30.0 mg/ml to about 35.0 mg/ml, about 35.0 mg/ml to about 40.0 mg/ml, about 40.0 mg/ml to about 45.0 mg/ml, or about 45.0 mg/ml to about 50.0 mg/ml. In other

embodiments, the liquid pharmaceutical composition comprises the antagonist anti-CD40 antibody or antigen-binding fragment thereof at a concentration of about 15.0 mg/ml, about 16.0 mg/ml, about 17.0 mg/ml, about 18.0 mg/ml, about 19.0 mg/ml, about 20.0 mg/ml, about 21.0 mg/ml, about 22.0 mg/ml, about 23.0 mg/ml, about 24.0 mg/ml, or  
5 about 25.0 mg/ml. The liquid pharmaceutical composition comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 antibody, or antigen-binding fragment thereof and a buffer that maintains the pH of the formulation in the range of about pH 5.0 to about pH 7.0, including about pH 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, and other such values within  
10 the range of about pH 5.0 to about pH 7.0. In some embodiments, the buffer maintains the pH of the formulation in the range of about pH 5.0 to about pH 6.5, about pH 5.0 to about pH 6.0, about pH 5.0 to about pH 5.5, about pH 5.5 to about 7.0, about pH 5.5 to about pH 6.5, or about pH 5.5 to about pH 6.0.

Any suitable buffer that maintains the pH of the liquid anti-CD40 antibody  
15 formulation in the range of about pH 5.0 to about pH 7.0 can be used in the formulation, so long as the physicochemical stability and desired biological activity of the antibody are retained as noted herein above. Suitable buffers include, but are not limited to, conventional acids and salts thereof, where the counter ion can be, for example, sodium, potassium, ammonium, calcium, or magnesium. Examples of conventional acids and  
20 salts thereof that can be used to buffer the pharmaceutical liquid formulation include, but are not limited to, succinic acid or succinate, citric acid or citrate, acetic acid or acetate, tartaric acid or tartarate, phosphoric acid or phosphate, gluconic acid or gluconate, glutamic acid or glutamate, aspartic acid or aspartate, maleic acid or maleate, and malic acid or malate buffers. The buffer concentration within the formulation can be from  
25 about 1 mM to about 50 mM, including about 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or other such values within the range of about 1 mM to about 50 mM. In some embodiments, the buffer concentration within the formulation is from about 5 mM to about 15 mM, including  
30 about 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, or other such values within the range of about 5 mM to about 15 mM.

In some embodiments of the invention, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof and succinate buffer or citrate buffer at a concentration that maintains the pH of the formulation in the range of about pH 5.0 to about pH 7.0, preferably about pH 5.0 to about pH 6.5. By "succinate buffer" or "citrate buffer" is intended a buffer comprising a salt of succinic acid or a salt of citric acid, respectively. In a preferred embodiment, the succinate or citrate counterion is the sodium cation, and thus the buffer is sodium succinate or sodium citrate, respectively. However, any cation is expected to be effective. Other possible succinate or citrate cations include, but are not limited to, potassium, ammonium, calcium, and magnesium. As noted above, the succinate or citrate buffer concentration within the formulation can be from about 1 mM to about 50 mM, including about 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or other such values within the range of about 1 mM to about 50 mM. In some embodiments, the buffer concentration within the formulation is from about 5 mM to about 15 mM, including about 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, or about 15 mM. In other embodiments, the liquid pharmaceutical formulation comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof at a concentration of about 0.1 mg/ml to about 50.0 mg/ml, or about 5.0 mg/ml to about 25.0 mg/ml, and succinate or citrate buffer, for example, sodium succinate or sodium citrate buffer, at a concentration of about 1 mM to about 20 mM, about 5 mM to about 15 mM, preferably about 10 mM.

Where it is desirable for the liquid pharmaceutical formulation to be near isotonic, the liquid pharmaceutical formulation comprising a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, and a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH 7.0 can further comprise an amount of an isotonicizing agent sufficient to render the formulation near isotonic. By "near isotonic" is intended the aqueous formulation has an osmolarity of about 240 mmol/kg to about 360 mmol/kg, preferably about 240 to about 340 mmol/kg,

more preferably about 250 to about 330 mmol/kg, even more preferably about 260 to about 320 mmol/kg, still more preferably about 270 to about 310 mmol/kg. Methods of determining the isotonicity of a solution are known to those skilled in the art. See, for example, Setnikar *et al.* (1959) *J. Am. Pharm. Assoc.* 48:628.

5           Those skilled in the art are familiar with a variety of pharmaceutically acceptable solutes useful in providing isotonicity in pharmaceutical compositions. The isotonizing agent can be any reagent capable of adjusting the osmotic pressure of the liquid pharmaceutical formulation of the present invention to a value nearly equal to that of a body fluid. It is desirable to use a physiologically acceptable isotonizing agent. Thus, the liquid pharmaceutical formulation comprising a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, and a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH 7.0, can further comprise components that can be used to provide isotonicity, for example, sodium chloride; amino acids such as alanine, valine, and glycine; sugars and sugar alcohols (polyols), including, but not limited to, glucose, dextrose, fructose, sucrose, maltose, mannitol, trehalose, glycerol, sorbitol, and xylitol; acetic acid, other organic acids or their salts, and relatively minor amounts of citrates or phosphates. The ordinary skilled person would know of additional agents that are suitable for providing optimal tonicity of the liquid formulation.

20           In some preferred embodiments, the liquid pharmaceutical formulation comprising a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, and a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH 7.0, further comprises sodium chloride as the isotonizing agent. 25 The concentration of sodium chloride in the formulation will depend upon the contribution of other components to tonicity. In some embodiments, the concentration of sodium chloride is about 50 mM to about 300 mM, about 50 mM to about 250 mM, about 50 mM to about 200 mM, about 50 mM to about 175 mM, about 50 mM to about 150 mM, about 75 mM to about 175 mM, about 75 mM to about 150 mM, about 100 mM to about 175 mM, about 100 mM to about 200 mM, about 100 mM to about 150 mM, about 125 mM to about 175 mM, about 125 mM to about 150 mM, about 130 mM to about 170

mM, about 130 mM to about 160 mM, about 135 mM to about 155 mM, about 140 mM to about 155 mM, or about 145 mM to about 155 mM. In one such embodiment, the concentration of sodium chloride is about 150 mM. In other such embodiments, the concentration of sodium chloride is about 150 mM, the buffer is sodium succinate or sodium citrate buffer at a concentration of about 5 mM to about 15 mM, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, and the formulation has a pH of about pH 5.0 to about pH 7.0, about pH 5.0 to about pH 6.0, or about pH 5.5 to about pH 6.5. In other embodiments, the liquid pharmaceutical formulation comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, at a concentration of about 0.1 mg/ml to about 50.0 mg/ml or about 5.0 mg/ml to about 25.0 mg/ml, about 150 mM sodium chloride, and about 10 mM sodium succinate or sodium citrate, at a pH of about pH 5.5.

Protein degradation due to freeze thawing or mechanical shearing during processing of a liquid pharmaceutical formulations of the present invention can be inhibited by incorporation of surfactants into the formulation in order to lower the surface tension at the solution-air interface. Thus, in some embodiments, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH 7.0, and further comprises a surfactant. In other embodiments, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH 7.0, an isotonicizing agent such as sodium chloride at a concentration of about 50 mM to about 300 mM, and further comprises a surfactant.

Typical surfactants employed are nonionic surfactants, including polyoxyethylene sorbitol esters such as polysorbate 80 (Tween 80) and polysorbate 20 (Tween 20); polyoxypropylene-polyoxyethylene esters such as Pluronic F68; polyoxyethylene

alcohols such as Brij 35; simethicone; polyethylene glycol such as PEG400; lysophosphatidylcholine; and polyoxyethylene-p-t-octylphenol such as Triton X-100.

Classic stabilization of pharmaceuticals by surfactants or emulsifiers is described, for example, in Levine *et al.* (1991) *J. Parenteral Sci. Technol.* 45(3):160-165, herein

5 incorporated by reference. A preferred surfactant employed in the practice of the present invention is polysorbate 80. Where a surfactant is included, it is typically added in an amount from about 0.001 % to about 1.0% (w/v), about 0.001% to about 0.5%, about 0.001% to about 0.4%, about 0.001% to about 0.3%, about 0.001% to about 0.2%, about 0.005% to about 0.5%, about 0.005% to about 0.2%, about 0.01% to about 0.5%, about 10 0.01% to about 0.2%, about 0.03% to about 0.5%, about 0.03% to about 0.3%, about 0.05% to about 0.5%, or about 0.05% to about 0.2%.

Thus, in some embodiments, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, the 15 buffer is sodium succinate or sodium citrate buffer at a concentration of about 1 mM to about 50 mM, about 5 mM to about 25 mM, or about 5 mM to about 15 mM; the formulation has a pH of about pH 5.0 to about pH 7.0, about pH 5.0 to about pH 6.0, or about pH 5.5 to about pH 6.5; and the formulation further comprises a surfactant, for example, polysorbate 80, in an amount from about 0.001% to about 1.0% or about 20 0.001% to about 0.5%. Such formulations can optionally comprise an isotonicizing agent, such as sodium chloride at a concentration of about 50 mM to about 300 mM, about 50 mM to about 200 mM, or about 50 mM to about 150 mM. In other embodiments, the liquid pharmaceutical formulation comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding 25 fragment thereof, at a concentration of about 0.1 mg/ml to about 50.0 mg/ml or about 5.0 mg/ml to about 25.0 mg/ml, including about 20.0 mg/ml; about 50 mM to about 200 mM sodium chloride, including about 150 mM sodium chloride; sodium succinate or sodium citrate at about 5 mM to about 20 mM, including about 10 mM sodium succinate or sodium citrate; sodium chloride at a concentration of about 50 mM to about 200 mM, 30 including about 150 mM; and optionally a surfactant, for example, polysorbate 80, in an amount from about 0.001% to about 1.0%, including about 0.001% to about 0.5%; where

the liquid pharmaceutical formulation has a pH of about pH 5.0 to about pH 7.0, about pH 5.0 to about pH 6.0, about pH 5.0 to about pH 5.5, about pH 5.5 to about pH 6.5, or about pH 5.5 to about pH 6.0.

5 The liquid pharmaceutical formulation can be essentially free of any preservatives and other carriers, excipients, or stabilizers noted herein above. Alternatively, the formulation can include one or more preservatives, for example, antibacterial agents, pharmaceutically acceptable carriers, excipients, or stabilizers described herein above provided they do not adversely affect the physicochemical stability of the antagonist anti-CD40 antibody or antigen-binding fragment thereof. Examples of acceptable carriers, 10 excipients, and stabilizers include, but are not limited to, additional buffering agents, co-solvents, surfactants, antioxidants including ascorbic acid and methionine, chelating agents such as EDTA, metal complexes (for example, Zn-protein complexes), and biodegradable polymers such as polyesters. A thorough discussion of formulation and selection of pharmaceutically acceptable carriers, stabilizers, and isomolytes can be found 15 in *Remington's Pharmaceutical Sciences* (18<sup>th</sup> ed.; Mack Publishing Company, Eaton, Pennsylvania, 1990), herein incorporated by reference.

After the liquid pharmaceutical formulation or other pharmaceutical composition described herein is prepared, it can be lyophilized to prevent degradation. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior 20 to use, the composition may be reconstituted with a sterile diluent (Ringer's solution, distilled water, or sterile saline, for example) that may include additional ingredients. Upon reconstitution, the composition is preferably administered to subjects using those methods that are known to those skilled in the art.

## 25 Use of Antagonist Anti-CD40 Antibodies in the Manufacture of Medicaments

The present invention also provides for the use of an antagonist anti-CD40 antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating an autoimmune disease and/or inflammatory disease in a subject, wherein the medicament is coordinated with treatment with at least one other therapy. By 30 "coordinated" is intended the medicament is to be used either prior to, during, or after treatment of the subject with at least one other therapy. Examples of other therapies

include, but are not limited to, those described herein above, i.e., surgery or surgical procedures (e.g. splenectomy, lymphadenectomy, thyroidectomy, plasmaphoresis, leukophoresis, cell, tissue, or organ transplantation, organ perfusion, intestinal procedures, and the like), radiation therapy, therapy such as steroid therapy and non-steroidal therapy, hormone therapy, cytokine therapy, therapy with dermatological agents (for example, topical agents used to treat skin conditions such as allergies, contact dermatitis, and psoriasis), immunosuppressive therapy, and other anti-inflammatory monoclonal antibody therapy, and the like, where treatment with the additional therapy, or additional therapies, occurs prior to, during, or subsequent to treatment of the subject with the medicament comprising the antagonist anti-CD40 antibody or antigen-binding fragment thereof, as noted herein above. In one such embodiment, the present invention provides for the use of the monoclonal antibody CHIR-12.12 or CHIR-5.9 in the manufacture of a medicament for treating an autoimmune disease and/or inflammatory disease in a subject, wherein the medicament is coordinated with treatment with at least one other therapy as noted herein above.

In some embodiments, the medicament comprising the antagonist anti-CD40 antibody, for example, the monoclonal antibody CHIR-12.12 or CHIR-5.9 disclosed herein, or antigen-binding fragment thereof is coordinated with treatment with two other therapies. Where the medicament comprising the antagonist anti-CD40 antibody is coordinated with two other therapies, use of the medicament can be prior to, during, or after treatment of the subject with either or both of the other therapies.

The invention also provides for the use of an antagonist anti-CD40 antibody, for example, the monoclonal antibody CHIR-12.12 or CHIR-5.9 disclosed herein, or antigen-binding fragment thereof in the manufacture of a medicament for treating an autoimmune disease and/or inflammatory disease in a subject, wherein the medicament is used in a subject that has been pretreated with at least one other therapy. By "pretreated" or "pretreatment" is intended the subject has been treated with one or more other therapies prior to receiving the medicament comprising the antagonist anti-CD40 antibody or antigen-binding fragment thereof. "Pretreated" or "pretreatment" includes subjects that have been treated with the other therapy, or other therapies, within 2 years, within 18 months, within 1 year, within 6 months, within 2 months, within 6 weeks, within 1



month, within 4 weeks, within 3 weeks, within 2 weeks, within 1 week, within 6 days, within 5 days, within 4 days, within 3 days, within 2 days, or even within 1 day prior to initiation of treatment with the medicament comprising the antagonist anti-CD40 antibody, for example, the monoclonal antibody CHIR-12.12 or CHIR-5.9 disclosed  
5 herein, or antigen-binding fragment thereof. It is not necessary that the subject was a responder to pretreatment with the prior therapy, or prior therapies. Thus, the subject that receives the medicament comprising the antagonist anti-CD40 antibody or antigen-binding fragment thereof could have responded, or could have failed to respond, to pretreatment with the prior therapy, or to one or more of the prior therapies where  
10 pretreatment comprised multiple therapies.

The following examples are offered by way of illustration and not by way of limitation.

## EXPERIMENTAL

### 15 *Introduction*

The antagonist anti-CD40 antibodies used in the examples below are CHIR-5.9 and CHIR-12.12. The CHIR-5.9 and CHIR-12.12 anti-CD40 antibodies are human IgG<sub>1</sub> subtype anti-human CD40 monoclonal antibodies (mAbs) generated by immunization of transgenic mice bearing the human IgG<sub>1</sub> heavy chain locus and the human K light chain  
20 locus (XenoMouse<sup>®</sup> technology (Abgenix; Fremont, California)). SF9 insect cells expressing CD40 extracellular domain were used as immunogen.

Briefly, splenocytes from immunized mice were fused with SP 2/0 or P 3 x 63Ag8.653 murine myeloma cells at a ratio of 10:1 using 50% polyethylene glycol as previously described by de Boer *et al.* (1988) *J. Immunol. Meth.* 113:143. The fused cells  
25 were resuspended in complete IMDM medium supplemented with hypoxanthine ( 0.1 mM), aminopterin ( 0.01 mM), thymidine ( 0.016 mM), and 0.5 ng/ml hIL-6 (Genzyme, Cambridge, Massachusetts). The fused cells were then distributed between the wells of 96-well tissue culture plates, so that each well contained 1 growing hybridoma on average.

30 After 10-14 days, the supernatants of the hybridoma populations were screened for specific antibody production. For the screening of specific antibody production by

the hybridoma clones, the supernatants from each well were pooled and tested for anti-CD40 activity specificity by ELISA first. The positives were then used for fluorescent cell staining of EBV-transformed B cells using a standard FACS assay. Positive hybridoma cells were cloned twice by limiting dilution in IMDM/FBS containing 0.5 ng/ml hIL-6.

A total of 31 mice spleens were fused with the mouse myeloma SP2/0 cells to generate 895 antibodies that recognize recombinant CD40 in ELISA. On average approximately 10% of hybridomas produced using Abgenix XenoMouse® technology (Abgenix; Fremont, California) may contain mouse lambda light chain instead of human kappa chain. The antibodies containing mouse light lambda chain were selected out. A subset of 260 antibodies that also showed binding to cell-surface CD40 were selected for further analysis. Stable hybridomas selected during a series of subcloning procedures were used for further characterization in binding and functional assays. For further details of the selection process, see copending provisional applications both entitled *"Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use,"* filed November 4, 2003, and November 26, 2003, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)), and 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), respectively; the contents of both of which are herein incorporated by reference in their entirety.

Clones from 7 other hybridomas were identified as having antagonistic activity. Based on their relative antagonistic potency and ADCC activities, two hybridoma clones were selected for further evaluation (Table 1 below). They are named 131.2F8.5.9 (5.9) and 153.8E2.D10.D6.12.12 (12.12).

Table 1. Summary of initial set of data with anti-CD40 IgG1 antibodies CHIR-5.9 and CHIR-12.12.

Mother Hybridoma	Hybridoma clones	cell surface binding	Antagonist	ADCC	CDC	CMCC#	V-region DNA sequence
131.2F5	131.2F5.8.5.9	+++	+++	++	-	12047	Yes
153.8E2	153.8E2D10D6.12.12	+++	+++	++++	-	12056	Yes

Mouse hybridoma line 131.2F8.5.9 (CMCC#12047) and hybridoma line 153.8E2.D10.D6.12.12 (CMCC#12056) have been deposited with the American Type Culture Collection (ATCC; 10801 University Blvd., Manassas, Virginia 20110-2209 (USA)) under Patent Deposit Number PTA-5542 and PTA-5543, respectively.

5       The cDNAs encoding the variable regions of the candidate antibodies were amplified by PCR, cloned, and sequenced. The amino acid sequences for the light chain and heavy chain of the CHIR-12.12 antibody are set forth in Figures 1A and 1B, respectively. See also SEQ ID NO:2 (light chain for mAb CHIR-12.12) and SEQ ID NO:4 (heavy chain for mAb CHIR-12.12). A variant of the heavy chain for mAb CHIR-12.12 is shown in Figure 1B (see also SEQ ID NO:5), which differs from SEQ ID NO:4 in having a serine residue substituted for the alanine residue at position 153 of SEQ ID NO:4. The nucleotide sequences encoding the light chain and heavy chain of the CHIR-12.12 antibody are set forth in Figures 2A and 2B, respectively. See also SEQ ID NO:1 (coding sequence for light chain for mAb CHIR-12.12) and SEQ ID NO:3 (coding  
10       sequence for heavy chain for mAb CHIR-12.12). The amino acid sequences for the light chain and heavy chain of the CHIR-5.9 antibody are set forth in Figures 3A and 3B, respectively. See also SEQ ID NO:6 (light chain for mAb CHIR-5.9) and SEQ ID NO:7 (heavy chain for mAb CHIR-5.9). A variant of the heavy chain for mAb CHIR-5.9 is shown in Figure 3B (see also SEQ ID NO:8), which differs from SEQ ID NO:7 in having  
15       a serine residue substituted for the alanine residue at position 158 of SEQ ID NO:7.  
20       

As expected for antibodies derived from independent hybridomas, there is substantial variation in the nucleotide sequences in the complementarity determining regions (CDRs). The diversity in the CDR3 region of  $V_H$  is believed to most significantly determine antibody specificity.

25       As shown by FACS analysis, CHIR-5.9 and CHIR-12.12 bind specifically to human CD40 and can prevent CD40-ligand binding. Both mAbs can compete off CD40-ligand pre-bound to cell surface CD40. The binding affinity of CHIR-5.9 to human CD40 is  $1.2 \times 10^{-8}$  M and the binding affinity of CHIR-12.12 to human CD40 is  $5 \times 10^{-10}$  M.

The CHIR-12.12 and CHIR-5.9 monoclonal antibodies are strong antagonists and  
30       inhibit *in vitro* CD40 ligand-mediated proliferation of normal B cells. For a more detailed description of these results, and the assays used to obtain them, see copending

provisional applications both entitled "*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*," filed November 4, 2003, and November 26, 2003, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)), and 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), respectively; the contents of both of which are herein incorporated by reference in their entirety.

Example 1: CHIR-12.12 Blocks CD40L-Mediated Cell Signaling

Soluble CD40 ligand (CD40L) activates B cells and induces various aspects of functional responses, including enhancement of survival and proliferation, and activation of NF $\kappa$ B, ERK/MAPK, PI3K/Akt, and p38 signaling pathways. In addition, CD40L-mediated CD40 stimulation provides survival signals by reduction of cleaved PARP and induction of the anti-apoptotic proteins, XIAP and Mcl-1, in normal B cells. CD40L-mediated CD40 stimulation also recruits TRAF2 and TRAF3 to bind CD40 cytoplasmic domain.

The following studies demonstrate that CHIR-12.12 directly inhibited all of these stimulation effects on normal human B cells. For example, CHIR-12.12 treatment resulted in increased cleavage of caspase-9, caspase-3, and PARP as well as reduction of XIAP and Mcl-1 in a time- and dose-dependent manner, restoring B cell apoptosis. Treatment with CHIR-12.12 also inhibited phosphorylation of I $\kappa$ B kinase (IKK)  $\alpha$  and  $\beta$  (NF $\kappa$ B pathway), ERK, Akt, and p38 in response to CD40L-mediated CD40 stimulation. Further, it was found that CHIR-12.12 did not trigger these apoptotic effects without initial CD40L-mediated CD40 stimulation.

*CHIR-12.12 inhibited survival mediated by CD40 ligand by inducing cleavage of PARP.*

In these experiments,  $0.6 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu$ g/ml sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). CHIR-12.12 (10  $\mu$ g/ml) and control IgG were then added. Cells were collected at 0, 20 minutes, 2 hours, 6 hours, 18 hours, and 26 hours. Cleaved caspase-9, cleaved caspase-3, cleaved PARP, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

Briefly, it was observed that CD40L-mediated CD40 stimulation provided survival signals as it did not result in increases of cleaved caspase-9, cleaved caspase-3, or cleaved PARP over time, indicating that the cells were not undergoing apoptosis. However, treatment with CHIR-12.12 resulted in an increase of these cleavage products, indicating that CHIR-12.12 treatment abrogated the effects of CD40L binding on survival signaling in sCD40L-stimulated normal B cells, restoring B cell apoptosis (data not shown).

*CHIR-12.12 inhibited expression of "survival" anti-apoptotic proteins.*

In these experiments,  $0.6 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu\text{g/ml}$  sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). CHIR-12.12 (10  $\mu\text{g/ml}$ ) and control IgG were then added. Cells were collected at 0, 20 minutes, 2 hours, 6 hours, 18 hours, and 26 hours. Mcl-1, XIAP, CD40, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

Briefly, sCD40L stimulation resulted in sustained expression of Mcl-1 and XIAP over time. However, treatment of the sCD40L-stimulated cells with CHIR 12.12 resulted in a decrease in expression of these proteins overtime (data not shown). Since Mcl-1 and XIAP are "survival" signals capable of blocking the apoptotic pathway, these results demonstrate that CHIR-12.12 treatment removes the blockade against apoptosis in sCD40L-stimulated normal B cells.

*CHIR-12.12 treatment inhibited phosphorylation of IKK $\alpha$  (Ser180) and IKK  $\beta$  (Ser 181) in normal B cells.*

In these experiments,  $1.0 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu\text{g/ml}$  sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). CHIR-12.12 (10  $\mu\text{g/ml}$ ) and control IgG were then added. Cells were collected at 0 and 20 minutes. Phosphorylated IKK $\alpha$  (Ser180) and IKK  $\beta$  (Ser 181) and total IKK $\beta$  controls were detected in cell lysates by Western blot.

Briefly, stimulation by sCD40L resulted in phosphorylation of IKK $\alpha$  (Ser 180) and IKK $\beta$  (Ser 181) over time; however, treatment with CHIR-12.12 abrogated this response to sCD40L stimulation in normal B cells (data not shown).

5 *CHIR-12.12 treatment inhibited survival mediated by CD40 ligand in a dose-dependent manner.*

In these experiments,  $0.6 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu\text{g/ml}$  sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). CHIR-12.12 (0.01, 0.1, 0.2, 0.5, 1.0  $\mu\text{g/ml}$ ) and control IgG were  
10 then added. Cells were collected at 24 hours. Cleaved PARP, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

Briefly, CHIR-12.12 treatment resulted in increase of PARP cleavage in sCD40L stimulated cells in a dose-dependent manner and therefore abrogated the survival signaling pathway in sCD40L-stimulated normal B cells (data not shown).

15

*CHIR-12.12 inhibited expression of "survival" anti-apoptotic proteins in a dose-dependent manner.*

In these experiments,  $0.6 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu\text{g/ml}$  sCD40L (Alexis Corp.,  
20 Bingham, Nottinghamshire, UK). CHIR-12.12 (0.5, 2, and 10  $\mu\text{g/ml}$ ) and control IgG were then added. Cells were collected at 22 hours. Mcl-1, XIAP, cleaved PARP, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

Briefly, CHIR-12.12 treatment reduced Mcl-1 and XIAP expression and increased cleaved PARP expression in sCD40L-stimulated cells in a dose-dependent manner, and  
25 thus abrogated these blockades to the apoptotic pathway in sCD40L-stimulated normal B cells (data not shown).

*CHIR-12.12 did not affect expression of anti-apoptotic proteins, cleaved-PARP, and XIAP, in the absence of soluble CD40L signaling.*

In these experiments,  $1.0 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were treated with CHIR-12.12 (10  $\mu\text{g}/\text{ml}$ ) and control IgG only (i.e., cells were not pre-stimulated with sCD40L before adding antibody). Cells were collected at 0, 4, 14, and 16 hours. XIAP, cleaved PARP, and  $\beta$ -actin controls were  
5 detected in cell lysates by Western blot.

Briefly, the results show that without sCD40L stimulation, the cells expressed increased concentrations of cleaved PARP, while expression of XIAP remained constant, in both IgG treated control cells and CHIR-12.12 cells (data not shown). These data indicate that CHIR-12.12 does not trigger apoptosis in normal human B cells without  
10 CD40L stimulation.

*CHIR-12.12 inhibits phosphorylation of IKK $\alpha$  (Ser180) and IKK $\beta$  (Ser181), Akt, ERK, and p38 in normal B cells.*

In these experiments,  $1.0 \times 10^6$  normal human B cells from healthy donors  
15 (percent purity between 85-95%) were serum starved in 1% FBS-containing media and stimulated with 1  $\mu\text{g}/\text{ml}$  sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). The cultures were treated with CHIR-12.12 (1 and 10  $\mu\text{g}/\text{ml}$ ) and control IgG. Cells were collected at 0 and 20 minutes. Phospho-IKK $\alpha$ , phospho-IKK $\beta$ , total IKK $\beta$ , phospho-ERK, total ERK, phospho-Akt, total Akt, phospho-p38, and total p38 were detected in  
20 cell lysates by Western blot.

Briefly, sCD40L stimulation resulted in increases in IKK $\alpha/\beta$  phosphorylation, ERK phosphorylation, Akt phosphorylation, and p38 phosphorylation, thus leading to survival and or proliferation of the cells. Treatment of the cells with CHIR-12.12 abrogated the effects of sCD40L stimulation on these signaling pathways in normal B  
25 cells (data not shown).

*CHIR 12.12 inhibits multiple signaling pathways such as PI3K and MEK /ERK in the CD40 signaling cascade.*

In these experiments,  $1.0 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were serum starved in 1% FBS-containing media and stimulated with 1  $\mu\text{g/ml}$  sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). The cultures were also treated with CHIR-12.12 (1 and 10  $\mu\text{g/ml}$ ), Wortmanin, (a PI3K/Akt inhibitor; 1 and 10  $\mu\text{M}$ ), LY 294002 (a PI3K/Akt inhibitor; 10 and 30  $\mu\text{M}$ ), and PD 98095 (a MEK inhibitor; 10 and 30  $\mu\text{g/ml}$ ). Cells were collected at 0 and 20 minutes. Phospho-ERK, phospho-Akt, total Akt, phospho-IKK $\alpha/\beta$ , and total were detected in cell lysates by Western blot.

Briefly, the results show that CHIR-12.12 abrogated the phosphorylation of all of these signal transduction molecules, whereas the signal transduction inhibitors showed only specific abrogation of signaling, indicating that CHIR-12.12 likely inhibits upstream of these signal transduction molecules mediated by CD40L stimulation (data not shown).

*CHIR-12.12 inhibits the binding of signaling molecules TRAF2 and TRAF3 to the cytoplasmic domain of CD40 in normal B cells.*

In these experiments,  $4.0 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were serum starved for four hours in 1% FBS-containing media and stimulated with 1  $\mu\text{g/ml}$  sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK) for 20 minutes. Cells were collected at 0 and 20 minutes. CD40 was immunoprecipitated using polyclonal anti-CD40 (Santa Cruz Biotechnology, CA), and was probed in a Western blot with anti-TRAF2 mAb (Santa Cruz Biotechnology, CA), anti-TRAF3 mAb (Santa Cruz Biotechnology, CA), and anti-CD40 mAb (Santa Cruz Biotechnology, CA).

Briefly, the results show that TRAF2 and TRAF3 co-precipitated with CD40 after sCD40L stimulation. In contrast, treatment with CHIR-12.12 abrogated formation of the CD40-TRAF2/3 signaling complex in sCD40L-stimulated normal B cells. There were no changes in CD40 expression (data not shown).

Without being bound by theory, the results of these experiments, and the results in the examples outlined above, indicate that the CHIR-12.12 antibody is a dual action antagonist anti-CD40 monoclonal antibody having a unique combination of attributes.



This fully human monoclonal antibody blocks CD40L-mediated CD40 signaling pathways for survival and proliferation of B cells; this antagonism leads to ultimate cell death. CHIR-12.12 also mediates recognition and binding by effector cells, initiating antibody dependent cellular cytotoxicity (ADCC). Once CHIR-12.12 is bound to effector cells, cytolytic enzymes are released, leading to B-cell apoptosis and lysis. CHIR-12.12 is a more potent anti-tumor antibody than is rituximab when compared in pre-clinical tumor models.

Example 2: CHIR-5.9 and CHIR-12.12 Bind to a Different Epitope on CD40 than 15B8

The candidate monoclonal antibodies CHIR-5.9 and CHIR-12.12 compete with each other for binding to CD40 but not with 15B8, an IgG<sub>2</sub> anti-CD40 mAb (see International Publication No. WO 02/28904). Antibody competition binding studies using Biacore were designed using CM5 biosensor chips with protein A immobilized via amine coupling, which was used to capture either anti-CD40, CHIR-12.12, or 15B8. Normal association/dissociation binding curves are observed with varying concentrations of CD40-his (data not shown). For competition studies, either CHIR-12.12 or 15B8 were captured onto the protein A surface. Subsequently a CD40-his / CHIR-5.9 Fab complex (100 nM CD40:1  $\mu$ M CHIR-5.9 Fab), at varying concentrations, was flowed across the modified surface. In the case of CHIR-12.12, there was no association of the complex observed, indicating CHIR-5.9 blocks binding of CHIR-12.12 to CD40-his. For 15B8, association of the Fab CHIR-5.9 complex was observed indicating CHIR-5.9 does not block binding of 15B8 to CD40 binding site. However, the off rate of the complex dramatically increased (data not shown).

It has also been determined that 15B8 and CHIR-12.12 do not compete for CD40-his binding. This experiment was set up by capturing CHIR-12.12 on the protein A biosensor chip, blocking residual protein A sites with control hIgG<sub>1</sub>, binding CD40-his and then flowing 15B8 over the modified surface. 15B8 did bind under these conditions indicating CHIR-12.12 does not block 15B8 from binding to CD40.

Example 3: Binding Properties of CHIR-12.12 and CHIR-5.9 mAb

Protein A was immobilized onto CM5 biosensor chips via amine coupling. Human anti-CD40 monoclonal antibodies, at 1.5 µg/ml, were captured onto the modified biosensor surface for 1.5 minutes at 10 µl/min. Recombinant soluble CD40-his was flowed over the biosensor surface at varying concentrations. Antibody and antigen were diluted in 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20 (HBS-EP). Kinetic and affinity constants were determined using the Biaevaluation software with a 1:1 interaction model/global fit.

As shown in Table 2 below, there is 121-fold difference in the off rate of CHIR-5.9 and CHIR-12.12 resulting in 24-fold higher affinity for CHIR-12.12.

Table 2. Summary of binding properties of CHIR-5.9 and CHIR-12.12 anti-CD40 antibodies.

<u>Antibody</u>	<u>K<sub>a</sub> (M<sup>-1</sup> sec<sup>-1</sup>))</u>	<u>k<sub>d</sub> (sec<sup>-1</sup>)</u>	<u>K<sub>D</sub> (nM)</u>
Anti-CD40, CHIR-5.9	$(12.35 \pm 0.64) \times 10^5$	$(15.0 \pm 1.3) \times 10^{-3}$	$12.15 \pm 0.35$
Anti-CD40, CHIR-12.12	$(2.41 \pm 0.13) \times 10^5$	$(1.24 \pm 0.06) \times 10^{-4}$	$0.51 \pm 0.02$

Example 4: Characterization of Epitope for Monoclonal Antibodies CHIR-12.12 andCHIR-5.9

To determine the location of the epitope on CD40 recognized by monoclonal antibodies CHIR-12.12 and CHIR-5.9, SDS-PAGE and Western blot analysis were performed. Purified CD40 (0.5 µg) was separated on a 4-12% NUPAGE gel under reducing and non-reducing conditions, transferred to PVDF membranes, and probed with monoclonal antibodies at 10 µg/ml concentration. Blots were probed with alkaline

phosphatase conjugated anti-human IgG and developed using the Western Blue<sup>R</sup> stabilized substrate for alkaline phosphatase (Promega).

Results indicate that anti-CD40 monoclonal antibody CHIR-12.12 recognizes epitopes on both the non-reduced and reduced forms of CD40, with the non-reduced form of CD40 exhibiting greater intensity than the reduced form of CD40 (Table 3; blots not shown). The fact that recognition was positive for both forms of CD40 indicates that this antibody interacts with a conformational epitope part of which is a linear sequence. Monoclonal antibody CHIR-5.9 primarily recognizes the non-reduced form of CD40 suggesting that this antibody interacts with a primarily conformational epitope (Table 3; blots not shown).

Table 3. Domain identification.

	Domain 1	Domain 2	Domain 3	Domain 4
mAb CHIR-12.12	-	+	-	-
mAb CHIR-5.9	-	+	-	-
mAb 15B8	+	-	-	-

To map the antigenic region on CD40, the four extracellular domains of CD40 were cloned and expressed in insect cells as GST fusion proteins. The secretion of the four domains was ensured with a GP67 secretion signal. Insect cell supernatant was analyzed by SDS-PAGE and western blot analysis to identify the domain containing the epitope.

Monoclonal antibody CHIR-12.12 recognizes an epitope on Domain 2 under both reducing and non-reducing conditions (Table 4; blots not shown). In contrast, monoclonal antibody CHIR-5.9 exhibits very weak recognition to Domain 2 (Table 4; blots not shown). Neither of these antibodies recognizes Domains 1, 3, or 4 in this analysis.

Table 4. Domain 2 analysis.

	Reduced	Non-reduced
mAb CHIR-12.12	++	+++
mAb CHIR-5.9	+	+

To define more precisely the epitope recognized by mAb CHIR-12.12, peptides were synthesized from the extracellular Domain 2 of CD40, which corresponds to the sequence PCGESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICT (residues 61-104 of the sequence shown in SEQ ID NO:10 or SEQ ID NO:12). SPOTs membranes (Sigma) containing thirty-five 10mer peptides with a 1-amino-acid offset were generated. Western blot analysis with mAb CHIR-12.12 and anti-human IgG beta-galactosidase as secondary antibody was performed. The blot was stripped and reprobed with mAb CHIR-5.9 to determine the region recognized by this antibody

SPOTs analysis probing with anti-CD40 monoclonal antibody CHIR-12.12 at 10 µg/ml yielded positive reactions with spots 18 through 22. The sequence region covered by these peptides is shown in Table 5.

Table 5. Results of SPOTs analysis probing with anti-CD40 monoclonal antibody CHIR-12.12.

Spot Number	Sequence Region
18	HQHKYCDPNL (residues 78-87 of SEQ ID NO:10 or 12)
19	QHKYCDPNLG (residues 79-88 of SEQ ID NO:10 or 12)
20	HKYCDPNLGL (residues 80-89 of SEQ ID NO:10 or 12)
21	KYCDPNLGLR (residues 81-90 of SEQ ID NO:10 or 12)
22	YCDPNLGLRV (residues 82-91 of SEQ ID NO:10 or 12)

These results correspond to a linear epitope of: YCDPNL (residues 82-87 of the sequence shown in SEQ ID NO:10 or SEQ ID NO:12). This epitope contains Y82, D84, and N86, which have been predicted to be involved in the CD40-CD40 ligand interaction.

SPOTs analysis with mAb CHIR-5.9 showed a weak recognition of peptides represented by spots 20-22 shown in Table 6, suggesting involvement of the region YCDPNLGL (residues 82-89 of the sequence shown in SEQ ID NO:10 or SEQ ID NO:12) in its binding to CD40. It should be noted that the mAbs CHIR-12.12 and CHIR-5.9 compete with each other for binding to CD40 in BIACORE analysis.

Table 6. Results of SPOTs analysis probing with anti-CD40 monoclonal antibody CHIR-5.9.

Spot Number	Sequence Region
20	HKYCDPNLGL (residues 80-89 of SEQ ID NO:10 or 12)
21	KYCDPNLGLR (residues 81-90 of SEQ ID NO:10 or 12)
22	YCDPNLGLRV (residues 82-91 of SEQ ID NO:10 or 12)

10

The linear epitopes identified by the SPOTs analyses are within the CD40 B1 module. The sequence of the CD40 B1 module is:

HKYCDPNLGLRVQQKGTSETDTIC (residues 80-103 of SEQ ID NO:10 or 12).

Within the linear epitope identified for CHIR-12.12 is C83. It is known that this cysteine residue forms a disulphide bond with C103. It is likely that the conformational epitope of the CHIR-12.12 mAb contains this disulfide bond (C83-C103) and/or surrounding amino acids conformationally close to C103.

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#### Example 5: Testing in Autoimmune and Inflammatory Disease Models

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##### *Systemic lupus erythematosus (SLE) model.*

CHIR-12.12 is tested in a model of human systemic lupus erythematosus (SLE) in which peripheral blood mononuclear cells (PMBCs) from SLE patients are engrafted into SCID mice. See, for example, the model described in Duchosal *et al.* (1990) *J. Exp.*

25 *Med.* 172:985-8.

After transfer of PBMCs from SLE patients into SCID mice, it is determined whether or not CHIR-12.12 treatment influences the T lymphocyte response to auto-antigen and auto-antibody production and disease manifestations such as glomerulonephritis. The first set of studies tests CHIR-12.12 as a single agent followed  
5 by testing the effect in a combination with other agents such as CTLA4-Ig.

*Multiple sclerosis model.*

Marmoset monkey experimental autoimmune encephalitis (EAE) is a model for human multiple sclerosis. See, for example, the model described in Raine *et al.* (1999)  
10 *Ann. Neurol.* 46:144-60 and Hart *et al.* (2004) *Lancet Neurol.* 3:588-97. CHIR-12.12 binds to marmoset CD40 and is tested for efficacy in this model.

*Inflammation and atherosclerosis.*

CHIR-12.12 is tested *in vitro* for its ability to inhibit CD40L-induced production  
15 of matrix-degrading enzymes, tissue factor expression, proinflammatory cytokines, and upregulation of adhesion molecules. Subsequent studies test the ability of CHIR-12.12 to show anti-inflammatory activities *in vivo* using transgenic mice expressing the human CD40 molecule. See, for example, the model described in Yasui (2002) *Int. Immunol.* 14:319-29.

20

*Transplantation.*

CHIR-12.12 is tested for its ability to prevent transplant rejection in non-human primate models. Cynomolgus monkey renal allograft recipients are treated with CHIR-12.12 antibody to demonstrate the effect on graft acceptance with or without additional  
25 immunosuppressive drugs such as cyclosporine, FK506, rapamycin, corticosteroids, CTLA4-Ig, and anti-B Lymphocyte Stimulator antibody, and the like.. See, the model described in Wee *et al.* (1992) *Transplantation* 53:501-7.

*Alzheimer's disease.*

30 CHIR-12.12 is tested first *in vitro* for its ability to block microglial activation. *In vivo* efficacy studies with CHIR-12.12 are conducted in double-transgenic mice

expressing human CD40 and overproducing amyloid-beta peptide. See, for example, the model described in Tan *et al.* (2002) *Nat. Neurosci.* 5:1288-93.

Example 6: Clinical Studies with CHIR-5.9 and CHIR-12.12

5 **Clinical Objectives**

The overall objective is to provide an effective therapy for rheumatoid arthritis (RA) by targeting cells with an antagonistic anti-CD40 IgG<sub>1</sub>. The signal for this disease is determined in phase I although some measure of activity may be obtained in phase I. Initially the agent is studied as a single agent, but will be combined with other therapeutic  
10 agents as development proceeds.

**Phase I**

- Evaluate safety and pharmacokinetics – dose escalation in subjects with RA.
- Choose dose based on safety, tolerability, and change in serum markers of CD40. In general an MTD is sought but other indications of efficacy (depletion of CD40<sup>+</sup>  
15 bearing cells, etc.) may be adequate for dose finding.
- Consideration of more than one dose, as some dose finding may be necessary in phase II.
- Patients are dosed weekly with real-time pharmacokinetic (Pk) sampling. Initially a 4-week cycle is the maximum dosing allowed. The Pk may be highly variable  
20 depending on the disease state, density of CD40 etc.
- This trial(s) is open to subjects with RA.
- Decision to discontinue or continue studies is based on safety, dose, and preliminary evidence of therapeutic activity.
- Activity of drug as determined by response rate is determined in Phase II.
- 25 • Identify dose(s) for Phase II.

**Phase II**

Several trials will be initiated in subjects with RA. More than one dose, and more than one schedule may be tested in a randomized phase II setting.

- Target a RA population that has failed current standard of care (nonsteroidal antiinflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs; e.g. gold and penicillamine) therapy failures)

5

- ✓ Decision to discontinue or continue with study is based on proof of therapeutic concept in Phase II
- ✓ Determine whether surrogate marker can be used as early indication of clinical efficacy
- ✓ Identify doses for Phase III

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### Phase III

Phase III will depend on where the signal is detected in phase II, and what competing therapies are considered to be the standard. If the signal is in a stage of disease where there is no standard of therapy, then a single arm, well-controlled study could serve as a pivotal trial. If there are competing agents that are considered standard, then head-to-head studies are conducted.

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### Example 7: Liquid Pharmaceutical Formulation for Antagonist Anti-CD40 Antibodies

The objective of this study was to investigate the effects of solution pH on stability of the antagonist anti-CD40 antibody CHIR-12.12 by both biophysical and biochemical methods in order to select the optimum solution environment for this antibody. Differential Scanning Calorimetry (DSC) results showed that the conformation stability of CHIR-12.12 is optimal in formulations having pH 5.5-6.5. Based on a combination of SDS-PAGE, Size-Exclusion HPLC (SEC-HPLC), and Cation-Exchange HPLC (CEX-HPLC) analysis, the physicochemical stability of CHIR-12.12 is optimal at about pH 5.0-5.5. In view of these results, one recommended liquid pharmaceutical formulation comprising this antibody is a formulation comprising CHIR-12.12 at about 20 mg/ml formulated in about 10 mM sodium succinate, about 150 mM sodium chloride, and having a pH of about pH 5.5.

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### Materials and Methods

The CHIR-12.12 antibody used in the formulation studies is a human monoclonal antibody produced by a CHO cell culture process. This MAb has a molecular weight of 150 kDa and consists of two light chains and two heavy chains linked together by disulfide bands. It is targeted against the CD40 cell surface receptor on CD40-expressing cells, including normal and malignant B cells, for treatment of various cancers and autoimmune/inflammatory diseases.

The anti-CD40 drug substance used for this study was a CHO-derived purified anti-CD40 (CHIR-12.12) bulk lot. The composition of the drug substance was 9.7 mg/ml CHIR-12.12 antibody in 10 mM sodium citrate, 150 mM sodium chloride, at pH 6.5. The control sample in the study was the received drug substance, followed by freezing at  $\leq -60^{\circ}\text{C}$ , thawing at RT and testing along with stability samples at predetermined time points. The stability samples were prepared by dialysis of the drug substance against different pH solutions and the CHIR-12.12 concentration in each sample was determined by UV 280 as presented in Table 7.

Table 7. CHIR-12.12 formulations.

Buffer Composition	pH	CHIR-12.12 Concentration ( mg/ml)
10 mM sodium citrate, 150 mM sodium chloride	4.5	9.0
10 mM sodium succinate, 150 mM sodium chloride	5.0	9.3
10 mM sodium succinate, 150 mM sodium chloride	5.5	9.2
10 mM sodium citrate, 150 mM sodium chloride	6.0	9.7
10 mM sodium citrate, 150 mM sodium chloride	6.5	9.4
10 mM sodium phosphate, 150 mM sodium chloride	7.0	9.4
10 mM sodium phosphate, 150 mM sodium chloride	7.5	9.5
10 mM glycine, 150 mM sodium chloride	9.0	9.5

Physicochemical stability of the CHIR-12.12 antibody in the various formulations was assayed using the following protocols.

#### *Differential Scanning Calorimetry (DSC)*

Conformational stability of different formulation samples was monitored using a MicroCal VP-DSC upon heating  $15^{\circ}\text{C}$  to  $90^{\circ}\text{C}$  at  $1^{\circ}\text{C}/\text{min}$ .

*SDS-PAGE*

Fragmentation and aggregation were estimated using 4-20% Tris-Glycine Gel  
5 under non-reducing and reducing conditions. Protein was detected by Coomassie blue staining.

*Size Exclusion Chromatograph (SEC-HPLC)*

10 Protein fragmentation and aggregation were also measured by a Water Alliance HPLC with a Tosohaas TSK-GEL 3000SWXL column, 100 mM sodium phosphate, pH 7.0 as mobile phase at a flow rate of 0.7 ml/min.

*Cation Exchange Chromatography (CEX-HPLC)*

15 Charge change related degradation was measured using Waters 600s HPLC system with a Dionex Propac WCX-10 column, 50 mM HEPES, pH 7.3 as mobile phase A and 50 mM HEPES containing 500 mM NaCl, pH 7.3 as mobile phase B at a flow rate of 0.5°C/min.

20

Results and Discussion*Conformational stability study.*

25 Thermal unfolding of CHIR-12.12 revealed at least two thermal transitions, probably representing unfolding melting of the Fab and the Fc domains, respectively. At higher temperatures, the protein presumably aggregated, resulting in loss of DSC signal. For the formulation screening purpose, the lowest thermal transition temperature was defined as the melting temperature,  $T_m$ , in this study. Figure 5 shows the thermal melting  
30 temperature as a function of formulation pHs. Formulations at pH 5.5-6.5 provided anti-CD40 with higher conformational stability as demonstrated by the higher thermal melting temperatures.

35

*SDS-PAGE analysis.*

The CHIR-12.12 formulation samples at pH 4.5-9.0 were incubated at 40°C for 2 months and subjected to SDS-PAGE analysis (data not shown). Under non-reducing conditions, species with molecular weight (MW) of 23 kDa and 27 kDa were observed in formulations above pH 5.5, and species with MW of 51 kDa were observed in all formulations, but appeared less at pH 5.0-5.5. A species with MW of 100 kDa could be seen at pH 7.5 and pH 9.0.

Under reducing conditions, CHIR-12.12 was reduced into free heavy chains and light chains with MW of 50 kDa and 24 kDa, respectively. The 100 kDa species seemed not fully reducible and increased with increasing solution pH, suggesting non-disulfide covalent association might occur in the molecules. Since there were other species with unknown identities on SDS-PAGE, stability comparison of each formulation is based on the remaining purity of CHIR-12.12. Formulations at pH 5.0-6.0 provided a more stable environment to CHIR-12.12. Few aggregates were detected by SDS-PAGE (data not shown).

#### *SEC-HPLC analysis .*

SEC-HPLC analysis detected the intact CHIR-12.12 as the main peak species, an aggregation species as a front peak species separate from the main peak species, a large fragment species as a shoulder peak on the back of the main peak species, and small fragment species were detected post-main peak species. After incubation at 5°C and 25°C for 3 months, negligible amounts of protein fragments and aggregates (<1.0% ) were detected in the above formulations and the CHIR-12.12 main peak species remained greater than 99% purity (data not shown). However, protein fragments gradually developed upon storage at 40°C and more fragments formed at pH 4.5 and pH 6.5-9.0, as shown in Table 8. After incubating the CHIR-12.12 formulations at 40°C for 3 months, about 2-3% aggregates were detected in pH 7.5 and pH 9.0, while less than 1% aggregates were detected in other pH formulations (data not shown). The SEC-HPLC results indicate CHIR-12.12 is more stable at about pH 5.0-6.0.

Table 8. SEC-HPLC results of CHIR-12.12 stability samples under real-time and accelerated storage conditions.

Sample	Main peak %				Fragments %			
	t=0	40°C 1 m	40°C 2 m	40°C 3 m	t=0	40°C 1 m	40°C 2 m	40°C 3 m
Control	99.4	99.2	99.9	99.5	<1.0	<1.0	<1.0	<1.0
pH 4.5	99.4	93.2	86.0	81.3	<1.0	6.4	13.2	18.1
pH 5.0	99.8	98.7	91.3	89.2	<1.0	<1.0	7.8	10.2
pH 5.5	99.8	98.9	91.4	90.6	<1.0	<1.0	7.6	8.8
pH 6.0	99.6	97.7	90.4	87.3	<1.0	1.9	8.2	11.7
pH 6.5	99.3	93.4	89.0	86.9	<1.0	5.6	9.9	12.4
pH 7.0	99.2	93.9	87.4	85.1	<1.0	5.5	11.1	13.5
pH 7.5	99.1	92.8	84.4	81.9	<1.0	6.4	12.9	16.2
pH 9.0	99.3	82.4	61.6	50.6	<1.0	15.4	36.2	47.6

5

*CEX-HPLC analysis.*

CEX-HPLC analysis detected the intact CHIR-12.12 as the main peak species, acidic variants eluted earlier than the main peak species, and C-terminal lysine addition variants eluted post-main peak species. Table 9 shows the dependence of the percentages of the remaining main peak CHIR-12.12 species and acidic variants on solution pH. The control sample already contained a high degree of acidic species (~33%), probably due to early-stage fermentation and purification processes. The susceptibility of CHIR-12.12 to higher pH solutions is evidenced by two facts. First, the initial formulation sample at pH 9.0 (t=0) already generated 12% more acidic species than the control. Second, the percentage of acidic species increased sharply with increasing pH. The charge change-related degradation is likely due to deamidation. The above data indicate that this type of degradation of CHIR-12.12 was minimized at about pH 5.0-5.5.

Table 9. Percentage of peak area by CEX-HPLC for CHIR-12.12 in different pH formulations under real-time and accelerated storage conditions.

Sample	Main peak %					Acidic variants %				
	t=0	5°C 3m	25°C 3 m	40°C 1 m	40°C 2 m	t=0	5°C 3m	25°C 3 m	40°C 1 m	40°C 2 m
Control	49.2	49.8	49.8	49.2	50.3	32.0	33.7	33.7	32.0	33.6
pH 4.5	48.5	49.7	43.7	39.7	30.0	32.5	32.6	38.0	44.2	56.4
pH 5.0	49.6	49.8	48.3	40.6	31.4	32.7	31.8	35.0	44.3	57.1

pH 5.5	50.7	50.3	48.1	40.0	30.2	32.6	31.8	37.8	48.9	63.3
pH 6.0	50.2	49.9	47.9	37.4	23.9	33.1	33.6	38.5	54.9	72.7
pH 6.5	49.4	49.9	42.3	29.7	14.6	33.3	33.6	47.7	65.2	84.6
pH 7.0	49.7	49.9	21.9	-	-	34.4	36.4	64.4	-	-
pH 7.5	49.3	48.3	12.7	-	-	35.5	40.1	79.2	-	-
pH 9.0	41.3	31.8	-	-	-	44.7	59.9	-	-	-

### Conclusion

The pH has a significant effect on conformational and physicochemical stabilities of CHIR-12.12. Charge change-related degradation was determined to be the main degradation pathway for CHIR-12.12, which was minimized at pH 5.0-5.5. Based on overall stability data, one recommended liquid pharmaceutical formulation comprising this antibody is a formulation comprising CHIR-12.12 at about 20 mg/ml formulated in about 10 mM sodium succinate, about 150 mM sodium chloride, and having a pH of about pH 5.5.

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims and list of embodiments disclosed herein. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.


All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

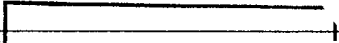
Applicant's or agent's file reference	PP23725.001	International application No.	PCT/US2004/
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 16, line 27	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depository institution American Type Culture Collection	
Address of depository institution (including postal code and country) 10801 University Blvd. Manassas, VA 20110-2209 USA	
Date of deposit 17 September 2003	Accession Number PTA-5542
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Page 20, line 27; Page 66, line 4; Page 87, line 22	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indicators are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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Authorized officer	



Applicant's or agent's file reference	PP23725.001	International application No.	PCT/US2004/
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<b>A.</b> The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 16, line 28	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depository institution <p align="center">American Type Culture Collection</p>	
Address of depository institution (including postal code and country) <p align="center">10801 University Blvd. Manassas, VA 20110-2209 USA</p>	
Date of deposit <p align="center">17 September 2003</p>	Accession Number <p align="center">PTA-5543</p>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Page 20, line 27; Page 66, line 4; Page 87, line 23	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indicators are not for all designated States)	
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# ATCC

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## BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

### INTERNATIONAL FORM

#### RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.

To: (Name and Address of Depositor or Attorney)

Chiron Corporation  
Attn: Karen Van Note  
4560 Horton Street  
Emeryville, CA 94608

Deposited on Behalf of: Chiron Corporation

#### Identification Reference by Depositor:

Mouze Hybridoma 131.2F8.5.9: CMCC#12047  
Mouze Hybridoma 153.8E2D10D6.12.12: CMCC#12056

#### Patent Deposit Designation

PTA-5542  
PTA-5543

The deposits were accompanied by:    a scientific description    a proposed taxonomic description indicated above. The deposits were received September 17, 2003 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: ☒ We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested September 23, 2003. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Mavis Harris  
Mavis Harris, Patent Specialist, ATCC Patent Depository

Date: October 2, 2003

cc: Lisa Alexander  
Ref: Docket or Case No.: P20107.001

RD49

Received Nov-03-03 03:20pm

From-510 923 4766

To-Intellectual Property Page 002



## THAT WHICH IS CLAIMED:

1. A method for treating a human subject for an inflammatory disease or autoimmune disease, comprising administering to said subject an effective amount of a human anti-CD40 monoclonal antibody that is capable of specifically binding to a human CD40 antigen expressed on the surface of a human CD40-expressing cell, said monoclonal antibody being free of significant agonist activity when bound to the CD40 antigen expressed on the surface of said cell, said human anti-CD40 monoclonal antibody being selected from the group consisting of:
- a) the monoclonal antibody CHIR-5.9 or CHIR-12.12;
  - b) the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;
  - c) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequence shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequence shown in SEQ ID NO:6 and SEQ ID NO:8;
  - d) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequence shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequence shown in SEQ ID NO:2 and SEQ ID NO:5;
  - e) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequence shown in SEQ ID NO:1 and SEQ ID NO:3;
  - f) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;
  - g) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

h) a monoclonal antibody that binds to an epitope comprising residues 82-89 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

i) a monoclonal antibody that competes with the monoclonal antibody CHIR-5.9 or CHIR-12.12 in a competitive binding assay;

5 j) the monoclonal antibody of preceding item a) or a monoclonal antibody of any one of preceding items c)-i), wherein said antibody is recombinantly produced; and

k) a monoclonal antibody that is an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-j), wherein said fragment retains the capability of specifically binding to said human CD40 antigen.

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2. The method of embodiment 1, wherein said monoclonal antibody binds to said human CD40 antigen with an affinity ( $K_D$ ) of at least about  $10^{-6}$  M to about  $10^{-12}$  M.

3. The method of embodiment 1, wherein said fragment is selected from the group consisting of a Fab fragment, an  $F(ab')_2$  fragment, an Fv fragment, and a single-chain Fv fragment.

4. The method of embodiment 1, wherein said inflammatory disease or autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), discoid lupus, lupus nephritis, sarcoidosis, juvenile arthritis, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, gouty arthritis, rejection of an organ or tissue transplant, graft versus host disease, multiple sclerosis, hyper IgE syndrome, polyarteritis nodosa, primary biliary cirrhosis, inflammatory bowel disease, Crohn's disease, celiac's disease (gluten-sensitive enteropathy), autoimmune hepatitis, pernicious anemia, autoimmune hemolytic anemia, psoriasis, scleroderma, myasthenia gravis, autoimmune thrombocytopenic purpura, autoimmune thyroiditis, Grave's disease, Hashimoto's thyroiditis, immune complex disease, chronic fatigue immune dysfunction syndrome (CFIDS), polymyositis and dermatomyositis, cryoglobulinemia, thrombolysis, cardiomyopathy, pemphigus vulgaris, pulmonary interstitial fibrosis, sarcoidosis, Type I and Type II diabetes mellitus, type 1, 2, 3, and 4 delayed-type hypersensitivity, allergy or allergic disorders, asthma, Churg-Strauss syndrome (allergic granulomatosis), atopic

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dermatitis, allergic and irritant contact dermatitis, urticaria, IgE-mediated allergy, atherosclerosis, vasculitis, idiopathic inflammatory myopathies, hemolytic disease, Alzheimer's disease, and chronic inflammatory demyelinating polyneuropathy.

- 5           5.       A method for treating a human subject for an inflammatory disease or autoimmune disease, comprising administering to said subject an effective amount of an antagonist anti-CD40 monoclonal antibody that specifically binds Domain 2 of human CD40 antigen, wherein said antibody is free of significant agonist activity when bound to Domain 2 of human CD40 antigen.
- 10           6.       The method of embodiment 5, wherein said antibody is a human antibody.
7.       The method of embodiment 5, wherein said antibody is recombinantly produced.
- 15           8.       The method of embodiment 5, wherein said antibody has the binding specificity of an antibody selected from the group consisting of the antibody produced by hybridoma cell line 5.9 and the antibody produced by hybridoma cell line 12.12.
- 20           9.       The method of embodiment 5, wherein said antibody is selected from the group consisting of the antibody produced by the hybridoma cell line deposited with the ATCC as Patent Deposit No. PTA-5542 and the antibody produced by the hybridoma cell line deposited with the ATCC as Patent Deposit No. PTA-5543.
- 25           10.      The method of embodiment 5, wherein said antibody has the binding specificity of monoclonal antibody CHIR-12.12 or CHIR-5.9.
11.      The method of embodiment 5, wherein said antibody binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or
- 30           SEQ ID NO:12.

12. The method of embodiment 5, wherein said antibody is selected from the group consisting of:

- a) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequence shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequence shown in SEQ ID NO:2 and SEQ ID NO:5;
- b) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequence shown in SEQ ID NO:1 and SEQ ID NO:3;
- c) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 12.12;
- d) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;
- e) a monoclonal antibody that competes with the monoclonal antibody CHIR-12.12 in a competitive binding assay;
- f) a monoclonal antibody of any one of preceding items a)-e), wherein said antibody is recombinantly produced; and
- g) a monoclonal antibody that is an antigen-binding fragment of the CHIR-12.12 monoclonal antibody or an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-f), where the fragment retains the capability of specifically binding to said human CD40 antigen.

13. The method of embodiment 5, wherein said inflammatory disease or autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), discoid lupus, lupus nephritis, sarcoidosis, juvenile arthritis, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, gouty arthritis, rejection of an organ or tissue transplant, graft versus host disease, multiple sclerosis, hyper IgE syndrome, polyarteritis nodosa, primary biliary cirrhosis, inflammatory bowel disease, Crohn's disease, celiac's disease (gluten-sensitive enteropathy), autoimmune hepatitis,

pernicious anemia, autoimmune hemolytic anemia, psoriasis, scleroderma, myasthenia  
gravis, autoimmune thrombocytopenic purpura, autoimmune thyroiditis, Grave's disease,  
Hashimoto's thyroiditis, immune complex disease, chronic fatigue immune dysfunction  
syndrome (CFIDS), polymyositis and dermatomyositis, cryoglobulinemia, thrombolysis,  
5 cardiomyopathy, pemphigus vulgaris, pulmonary interstitial fibrosis, sarcoidosis, Type I  
and Type II diabetes mellitus, type 1, 2, 3, and 4 delayed-type hypersensitivity, allergy or  
allergic disorders, asthma, Churg-Strauss syndrome (allergic granulomatosis), atopic  
dermatitis, allergic and irritant contact dermatitis, urticaria, IgE-mediated allergy,  
atherosclerosis, vasculitis, idiopathic inflammatory myopathies, hemolytic disease,  
10 Alzheimer's disease, and chronic inflammatory demyelinating polyneuropathy.

14. A method for treating a human subject for transplant rejection, comprising  
administering to said subject an effective amount of a human anti-CD40 monoclonal  
antibody that is capable of specifically binding to a human CD40 antigen expressed on  
15 the surface of a human CD40-expressing cell, said monoclonal antibody being free of  
significant agonist activity when bound to the CD40 antigen expressed on the surface of  
said cell, said human anti-CD40 monoclonal antibody being selected from the group  
consisting of:

- a) the monoclonal antibody CHIR-5.9 or CHIR-12.12;
- 20 b) the monoclonal antibody produced by the hybridoma cell line 5.9 or  
12.12;
- c) a monoclonal antibody comprising an amino acid sequence selected from  
the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in  
SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequence shown in SEQ  
25 ID NO:6 and SEQ ID NO:7, and both the sequence shown in SEQ ID NO:6 and SEQ ID  
NO:8;
- d) a monoclonal antibody comprising an amino acid sequence selected from  
the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in  
SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequence shown in SEQ  
30 ID NO:2 and SEQ ID NO:4, and both the sequence shown in SEQ ID NO:2 and SEQ ID  
NO:5;

- e) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequence shown in SEQ ID NO:1 and SEQ ID NO:3;
- 5 f) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;
- g) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;
- h) a monoclonal antibody that binds to an epitope comprising residues 82-89  
10 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;
- i) a monoclonal antibody that competes with the monoclonal antibody CHIR-5.9 or CHIR-12.12 in a competitive binding assay;
- j) the monoclonal antibody of preceding item a) or a monoclonal antibody of any one of preceding items c)-i), wherein said antibody is recombinantly produced; and
- 15 k) a monoclonal antibody that is an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-j), wherein said fragment retains the capability of specifically binding to said human CD40 antigen.

15. The method of embodiment 14, wherein said treatment further comprises  
20 administering an immunosuppressive agent in a pharmaceutically acceptable excipient.

16. The method of claim 15, wherein the immunosuppressive agent is selected from the group consisting of cyclosporine, FK506, rapamycin, corticosteroids, CTLA4-Ig, and anti-B Lymphocyte Stimulator antibody.

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17. A method for treating a human subject for rheumatoid arthritis, comprising administering to said subject an effective amount of a human anti-CD40 monoclonal antibody that is capable of specifically binding to a human CD40 antigen expressed on the surface of a human CD40-expressing cell, said monoclonal antibody  
30 being free of significant agonist activity when bound to the CD40 antigen expressed on

the surface of said cell, said human anti-CD40 monoclonal antibody being selected from the group consisting of:

- a) the monoclonal antibody CHIR-5.9 or CHIR-12.12;
- b) the monoclonal antibody produced by the hybridoma cell line 5.9 or  
5 12.12;
- c) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequence shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequence shown in SEQ ID NO:6 and SEQ ID  
10 NO:8;
- d) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequence shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequence shown in SEQ ID NO:2 and SEQ ID  
15 NO:5;
- e) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequence shown in SEQ ID NO:1 and SEQ ID NO:3;
- 20 f) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;
- g) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;
- h) a monoclonal antibody that binds to an epitope comprising residues 82-89  
25 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;
- i) a monoclonal antibody that competes with the monoclonal antibody CHIR-5.9 or CHIR-12.12 in a competitive binding assay;
- j) the monoclonal antibody of preceding item a) or a monoclonal antibody of any one of preceding items c)-i), wherein said antibody is recombinantly produced; and

k) a monoclonal antibody that is an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-j)), wherein said fragment retains the capability of specifically binding to said human CD40 antigen; and further administering an immunosuppressive agent in a pharmaceutically acceptable excipient.

18. The method of claim 17, wherein the immunosuppressive agent is selected from the group consisting of cyclosporine, FK506, rapamycin, corticosteroids, CTLA4-Ig, an anti-CD20 antibody, and anti-B Lymphocyte Stimulator antibody.

19. A method for treating a human subject having an inflammatory disease or autoimmune disease comprising administering to said subject an effective amount of a human monoclonal antibody that is capable of specifically binding to CD40 antigen, said monoclonal antibody being free of significant agonistic activity, wherein said antibody is the monoclonal antibody CHIR-5.9 or CHIR-12.12.



**FIGURE 1A**CHIR 12.12 light chain:

leader:

MALPAQLLGLLMLWVSGSSG

variable:

DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLISLGSNRASG  
VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFGPGTKVDIR

constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK  
DSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**FIGURE 1B**CHIR-12.12 heavy chain:

leader:

MEFGLSWVFLVAILRGVQC

variable:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYEESNRYHAD  
SVKGRFTISRDN SKITLYLQMNSLRTEDTAVYYCARDGGIAAPGPDYWGQGTLVTVSS

constant:

ASTKGPSVFPLAPASKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL  
YSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF  
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL  
TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV  
MHEALHNHYTQKSLSLSPGK

alternative constant region:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL  
YSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF  
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL  
TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV  
MHEALHNHYTQKSLSLSPGK

**FIGURE 2A**

DNA sequence of light chain of CHIR-12.12:

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agcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagctcaacaggg  
gagagtgttag3'

**FIGURE 2B**

DNA sequence of heavy chain of CHIR-12.12 (including introns):

5'atggagtttgggctgagctgggtttccttggtgctattttaagaggtgtccagtgtcaggtgcagttggtggagtctgggggaggcgt  
ggtccagcctgggaggtccctgagactctcctgtgcagcctctggattcaccttcagtagctatggcatgactgggtccgccaggctc  
caggcaaggggctggagtgggtggcagttatatcatatgaggaaagtaatagataccatgcagactccgtgaaggggccgattacca  
tctccagagacaattccaagatcacgctgtatctgcaaatgaacagcctcagaactgaggacacggctgtgtattactgtgcgagagat  
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acaagagagattggtgagaggccagcagaggaggagggtgtctgctggaagccaggctcagcgtcctgctggacgcacatccc  
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aagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcc  
ctgtctccgggtaaatga3'

**FIGURE 3A**CHIR-5.9 light chain:

leader:

MALLAQLLGLLMLWVPGSSG

variable:

AIVMTQPPLSSPVTLGQPASISCRSSQSLVHSDGNTYLNWLQQRPGQPPRLLIYKFFRRLSG  
VPDRFSGSGAGTDFTLKISRVEADVGVYYCMQVTQFPHTFGQGTRLEIK

constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK  
DSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**FIGURE 3B**CHIR-5.9 heavy chain:

leader:

MGSTAILALLLAVLQGVCA

variable:

EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTRYSP  
SFQGGQVTISADKSISTAYLQWSSLKASDTAMYICARGTAAGR DYYYYYGM DVWGQGTTTVTS  
S

constant:

ASTKGPSVFPLAPASKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL  
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF  
LFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL  
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV  
MHEALHNHYTQKSLSLSPGK

alternative constant region:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL  
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF  
LFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL  
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV  
MHEALHNHYTQKSLSLSPGK

**FIGURE 4A**

Coding sequence for short isoform of human CD40:

```
1 atggttcgtc tgcctctgca gtgcgtctc tggggctgct tgctgaccgc tgtccatcca
61 gaaccaccca ctgcatgcag agaaaaacag tacctaataa acagtcagtg ctgttcttg
121 tgccagccag gacagaaact ggtgagtgac tgcacagagt tcaactgaaac ggaatgcctt
181 ccttgcggtg aaagcgaatt cctagacacc tggaacagag agacacactg ccaccagcac
241 aaatactgcg accccaacct agggcttcgg gtccagcaga agggcacctc agaaacagac
301 accatctgca cctgtgaaga aggtggcac tgtacgagtg aggcctgtga gagctgtgtc
361 ctgcaccgct catgctcgcc cggctttggg gtcaagcaga ttgctacagg ggtttctgat
421 accatctgcg agccctgccc agtcggcttc ttctcaatg tgtcatctgc ttctgaaaaa
481 tgtcacctt ggacaaggtc ccaggatcg gctgagagcc ctggtggtga tccccatcat
541 cttegggatc ctgttgcca tcctcttggg gctggtcttt atcaaaaagg tggccaagaa
601 gccaaccaat aa
```

**FIGURE 4B**

Encoded short isoform of human CD40:

```
1 mvrplqcvl wgciltavhp epptacrekq ylinsqccsl cpggqklvsd cteftetecf
61 pcgesefldt wnrethchqh kyedpnlgrr vqqkgtsetd tictceegwh ctseacescv
121 lhrscspgfg vkqiatgvsd ticepcpvgf fsnvssafek chpwtrspgs aespaggdphh
181 lrdpvchplg aglyqkggqe anq
```

**FIGURE 4C**

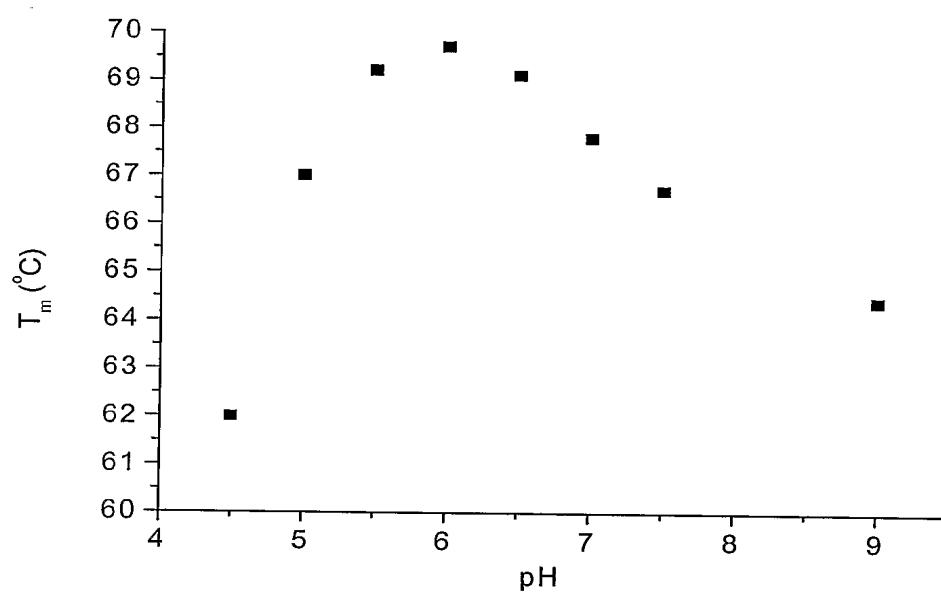
Coding sequence for long isoform of human CD40:

```
1 atggttcgtc tgcctctgca gtgcgtctc tggggctgct tgctgaccgc tgtccatcca
61 gaaccaccca ctgcatgcag agaaaaacag tacctaataa acagtcagtg ctgttcttg
121 tgccagccag gacagaaact ggtgagtgac tgcacagagt tcaactgaaac ggaatgcctt
181 ccttgcgggtg aaagcgaatt cctagacacc tggaacagag agacacactg ccaccagcac
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421 accatctgcg agccctgccc agtcggcttc ttctccaatg tgcactctgc ttctgaaaaa
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661 aagccaacca ataaggcccc ccacccaag caggaacccc aggagatcaa tttcccgac
721 gatcttctg gctccaacac tgctgtcca gtgcaggaga ctttacatgg atgccaaccg
781 gtcaccagg aggatggcaa agagagtgc atctcagtc aggagagaca gtga
```

**FIGURE 4D**

Encoded long isoform of human CD40:

```
1 mvrplqcvl wgclltavhp epptacrekq ylinsqccsl cqpqqklvsd cteftetec1
61 pcgesefldt wnrethchqh kydpnlglr vqqkgtsetd tictceegwh ctseacescv
121 lhrscspgfg vkqiatgvsd ticepcpvgf fsnvssafek chpwtscetk dlvvqqagtn
181 ktdvvcgpqd rlravvipi ifgilfaill vlvfikkvak kptnkaphpk qepqeinfpd
241 dlpgsntaap vqetlhgcqp vtqedgkesr isvqerq
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**FIGURE 5**

## SEQUENCE LISTING

<110> Long, Li  
 Luqman, Mohammad  
 Yabannavar, Asha  
 Zaror, Isabel

<120> USE OF ANTAGONISTIC ANTI-CD40 ANTIBODIES  
 FOR TREATMENT OF AUTOIMMUNE AND INFLAMMATORY DISEASE AND ORGAN  
 TRANSPLANT REJECTION

<130> PP23725.001 (284072)

<150> 60/565,710

<151> 2004-04-27

<150> 60/525,579

<151> 2003-11-26

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 human anti-CD40 antibody

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1 5 10 15	
gga tcc agt ggg gat att gtg atg act cag tct cca ctc tcc ctg acc	96
Gly Ser Ser Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Thr	
20 25 30	
gtc acc cct gga gag ccg gcc tcc atc tcc tgc agg tcc agt cag agc	144
Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser	
35 40 45	
ctc ctg tat agt aat gga tac aac tat ttg gat tgg tac ctg cag aag	192
Leu Leu Tyr Ser Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys	
50 55 60	
cca ggg cag tct cca cag gtc ctg atc tct ttg ggt tct aat cgg gcc	240
Pro Gly Gln Ser Pro Gln Val Leu Ile Ser Leu Gly Ser Asn Arg Ala	
65 70 75 80	
tcc ggg gtc cct gac agg ttc agt ggc agt gga tca ggc aca gat ttt	288
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe	
85 90 95	
aca ctg aaa atc agc aga gtg gag gct gag gat gtt ggg gtt tat tac	336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr	

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Cys	Met	Gln	Ala	Arg	Gln	Thr	Pro	Phe	Thr	Phe	Gly	Pro	Gly	Thr	Lys					
115						120					125									
gtg	gat	atc	aga	cga	act	gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	432				
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130						135					140									
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Pro	Ser	Asp	Glu	Gln	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu						
145						150					155					160				
ctg	aat	aac	ttc	tat	ccc	aga	gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	528				
Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp					
165						170					175									
aac	gcc	ctc	caa	tcg	ggg	aac	tcc	cag	gag	agt	gtc	aca	gag	cag	gac	576				
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agc	aag	gac	agc	acc	tac	agc	ctc	agc	agc	acc	ctg	acg	ctg	agc	aaa	624				
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210						215					220									
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225						230					235									

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&lt;211&gt; 239

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Light chain of CHIR-12.12 human anti-CD40 antibody

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			20					25					30		
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			35				40					45			
Leu	Leu	Tyr	Ser	Asn	Gly	Tyr	Asn	Tyr	Leu	Asp	Trp	Tyr	Leu	Gln	Lys
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			115				120					125			
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	145				150					155				160	
Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp



				165					170					175	
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			180					185					190		
Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys
		195					200						205		
Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln
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Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	
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 human anti-CD40 antibody (with introns)

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<210> 4  
 <211> 469  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Heavy chain of CHIR-12.12 human anti-CD40 antibody

<400> 4  
 Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Arg Gly

1	5	10	15
Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln			
20	25	30	
Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe			
35	40	45	
Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu			
50	55	60	
Glu Trp Val Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala			
65	70	75	80
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile			
85	90	95	
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val			
100	105	110	
Tyr Tyr Cys Ala Arg Asp Gly Gly Ile Ala Ala Pro Gly Pro Asp Tyr			
115	120	125	
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly			
130	135	140	
Pro Ser Val Phe Pro Leu Ala Pro Ala Ser Lys Ser Thr Ser Gly Gly			
145	150	155	160
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val			
165	170	175	
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe			
180	185	190	
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val			
195	200	205	
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val			
210	215	220	
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys			
225	230	235	240
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu			
245	250	255	
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr			
260	265	270	
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val			
275	280	285	
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val			
290	295	300	
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser			
305	310	315	320
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu			
325	330	335	
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala			
340	345	350	
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro			
355	360	365	
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln			
370	375	380	
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala			
385	390	395	400
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr			
405	410	415	
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu			
420	425	430	
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser			
435	440	445	
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser			
450	455	460	
Leu Ser Pro Gly Lys			
465			

&lt;210&gt; 5

&lt;211&gt; 469

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Heavy chain of variant of CHIR-12.12 human  
anti-CD40 antibody

&lt;400&gt; 5

```

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Arg Gly
 1      5      10      15
Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
 20      25      30
Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 35      40      45
Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 50      55      60
Glu Trp Val Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala
 65      70      75      80
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile
 85      90      95
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val
100      105      110
Tyr Tyr Cys Ala Arg Asp Gly Gly Ile Ala Ala Pro Gly Pro Asp Tyr
115      120      125
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
130      135      140
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145      150      155      160
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
165      170      175
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
180      185      190
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
195      200      205
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
210      215      220
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
225      230      235      240
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
245      250      255
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
260      265      270
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
275      280      285
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
290      295      300
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305      310      315      320
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
325      330      335
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
340      345      350
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
355      360      365
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
370      375      380
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385      390      395      400
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
405      410      415
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
420      425      430
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
435      440      445
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
450      455      460
Leu Ser Pro Gly Lys
465

```

<210> 6  
 <211> 239  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Light chain of CHIR-5.9 human anti-CD40 antibody

<400> 6  
 Met Ala Leu Leu Ala Gln Leu Leu Gly Leu Leu Met Leu Trp Val Pro  
 1 5 10 15  
 Gly Ser Ser Gly Ala Ile Val Met Thr Gln Pro Pro Leu Ser Ser Pro  
 20 25 30  
 Val Thr Leu Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser  
 35 40 45  
 Leu Val His Ser Asp Gly Asn Thr Tyr Leu Asn Trp Leu Gln Gln Arg  
 50 55 60  
 Pro Gly Gln Pro Pro Arg Leu Leu Ile Tyr Lys Phe Phe Arg Arg Leu  
 65 70 75 80  
 Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ala Gly Thr Asp Phe  
 85 90 95  
 Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr  
 100 105 110  
 Cys Met Gln Val Thr Gln Phe Pro His Thr Phe Gly Gln Gly Thr Arg  
 115 120 125  
 Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro  
 130 135 140  
 Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu  
 145 150 155 160  
 Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp  
 165 170 175  
 Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp  
 180 185 190  
 Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys  
 195 200 205  
 Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln  
 210 215 220  
 Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 225 230 235

<210> 7  
 <211> 474  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Heavy chain of CHIR-5.9 human anti-CD40 antibody

<400> 7  
 Met Gly Ser Thr Ala Ile Leu Ala Leu Leu Leu Ala Val Leu Gln Gly  
 1 5 10 15  
 Val Cys Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
 20 25 30  
 Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe  
 35 40 45  
 Thr Ser Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu  
 50 55 60  
 Glu Trp Met Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser  
 65 70 75 80  
 Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser  
 85 90 95  
 Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met  
 100 105 110  
 Tyr Tyr Cys Ala Arg Gly Thr Ala Ala Gly Arg Asp Tyr Tyr Tyr Tyr

```

      115      120      125
Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 130      135      140
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ala Ser Lys
 145      150      155      160
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
      165      170      175
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
      180      185      190
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
      195      200      205
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
      210      215      220
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 225      230      235      240
Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
      245      250      255
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
      260      265      270
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
      275      280      285
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
      290      295      300
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 305      310      315      320
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
      325      330      335
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
      340      345      350
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
      355      360      365
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
      370      375      380
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 385      390      395      400
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
      405      410      415
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
      420      425      430
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
      435      440      445
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
      450      455      460
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465      470

```

&lt;210&gt; 8

&lt;211&gt; 474

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Heavy chain of variant CHIR-5.9 human anti-CD40 antibody

&lt;400&gt; 8

```

Met Gly Ser Thr Ala Ile Leu Ala Leu Leu Leu Ala Val Leu Gln Gly
 1      5      10      15
Val Cys Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
      20      25      30
Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe
      35      40      45
Thr Ser Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu
      50      55      60
Glu Trp Met Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser

```

```

65          70          75          80
Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser
      85          90          95
Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met
      100        105        110
Tyr Tyr Cys Ala Arg Gly Thr Ala Ala Gly Arg Asp Tyr Tyr Tyr Tyr
      115        120        125
Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
      130        135        140
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
      145        150        155
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
      165        170        175
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
      180        185        190
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
      195        200        205
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
      210        215        220
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
      225        230        235
Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
      245        250        255
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
      260        265        270
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
      275        280        285
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
      290        295        300
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
      305        310        315
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
      325        330        335
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
      340        345        350
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
      355        360        365
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
      370        375        380
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
      385        390        395
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
      405        410        415
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
      420        425        430
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
      435        440        445
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
      450        455        460
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465          470

```

```

<210> 9
<211> 612
<212> DNA
<213> Homo sapiens

```

```

<220>
<221> CDS
<222> (1)...(612)

<221> misc_feature
<222> (0)...(0)
<223> Coding sequence for short isoform of human CD40

```

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<400> 9
atg gtt cgt ctg cct ctg cag tgc gtc ctc tgg ggc tgc ttg ctg acc 48
Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
1 5 10 15

gct gtc cat cca gaa cca ccc act gca tgc aga gaa aaa cag tac cta 96
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
20 25 30

ata aac agt cag tgc tgt tct ttg tgc cag cca gga cag aaa ctg gtg 144
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
35 40 45

agt gac tgc aca gag ttc act gaa acg gaa tgc ctt cct tgc ggt gaa 192
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
50 55 60

agc gaa ttc cta gac acc tgg aac aga gag aca cac tgc cac cag cac 240
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
65 70 75 80

aaa tac tgc gac ccc aac cta ggg ctt cgg gtc cag cag aag ggc acc 288
Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
85 90 95

tca gaa aca gac acc atc tgc acc tgt gaa gaa ggc tgg cac tgt acg 336
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
100 105 110

agt gag gcc tgt gag agc tgt gtc ctg cac cgc tca tgc tcg ccc ggc 384
Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly
115 120 125

ttt ggg gtc aag cag att gct aca ggg gtt tct gat acc atc tgc gag 432
Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu
130 135 140

ccc tgc cca gtc ggc ttc ttc tcc aat gtg tca tct gct ttc gaa aaa 480
Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
145 150 155 160

tgt cac cct tgg aca agg tcc cca gga tcg gct gag agc cct ggt ggt 528
Cys His Pro Trp Thr Arg Ser Pro Gly Ser Ala Glu Ser Pro Gly Gly
165 170 175

gat ccc cat cat ctt cgg gat cct gtt tgc cat cct ctt ggt gct ggt 576
Asp Pro His His Leu Arg Asp Pro Val Cys His Pro Leu Gly Ala Gly
180 185 190

ctt tat caa aaa ggt ggc caa gaa gcc aac caa taa 612
Leu Tyr Gln Lys Gly Gly Gln Glu Ala Asn Gln *
195 200

```

```

<210> 10
<211> 203
<212> PRT
<213> Homo sapiens

```

```

<400> 10
Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
1 5 10 15
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
20 25 30
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
35 40 45

```

```

Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
 50          55          60
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
 65          70          75          80
Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
          85          90          95
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
          100          105          110
Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly
          115          120          125
Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu
 130          135          140
Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
 145          150          155          160
Cys His Pro Trp Thr Arg Ser Pro Gly Ser Ala Glu Ser Pro Gly Gly
          165          170          175
Asp Pro His His Leu Arg Asp Pro Val Cys His Pro Leu Gly Ala Gly
          180          185          190
Leu Tyr Gln Lys Gly Gly Gln Glu Ala Asn Gln
          195          200

```

&lt;210&gt; 11

&lt;211&gt; 834

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)...(834)

&lt;221&gt; misc\_feature

&lt;222&gt; (0)...(0)

&lt;223&gt; Coding sequence for long isoform of human CD40

&lt;400&gt; 11

```

atg gtt cgt ctg cct ctg cag tgc gtc ctc tgg ggc tgc ttg ctg acc 48
Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
 1          5          10          15

gct gtc cat cca gaa cca ccc act gca tgc aga gaa aaa cag tac cta 96
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
          20          25          30

ata aac agt cag tgc tgt tct ttg tgc cag cca gga cag aaa ctg gtg 144
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
          35          40          45

agt gac tgc aca gag ttc act gaa acg gaa tgc ctt cct tgc ggt gaa 192
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
          50          55          60

agc gaa ttc cta gac acc tgg aac aga gag aca cac tgc cac cag cac 240
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
          65          70          75          80

aaa tac tgc gac ccc aac cta ggg ctt cgg gtc cag cag aag ggc acc 288
Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
          85          90          95

tca gaa aca gac acc atc tgc acc tgt gaa gaa ggc tgg cac tgt acg 336
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
          100          105          110

agt gag gcc tgt gag agc tgt gtc ctg cac cgc tca tgc tcg ccc ggc 384
Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly

```



115	120	125	
ttt ggg gtc aag cag att gct aca ggg gtt tct gat acc atc tgc gag Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu 130 135 140			432
ccc tgc cca gtc ggc ttc ttc tcc aat gtg tca tct gct ttc gaa aaa Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys 145 150 155 160			480
tgt cac cct tgg aca agc tgt gag acc aaa gac ctg gtt gtg caa cag Cys His Pro Trp Thr Ser Cys Glu Thr Lys Asp Leu Val Val Gln Gln 165 170 175			528
gca ggc aca aac aag act gat gtt gtc tgt ggt ccc cag gat cgg ctg Ala Gly Thr Asn Lys Thr Asp Val Val Cys Gly Pro Gln Asp Arg Leu 180 185 190			576
aga gcc ctg gtg gtg atc ccc atc atc ttc ggg atc ctg ttt gcc atc Arg Ala Leu Val Val Ile Pro Ile Ile Phe Gly Ile Leu Phe Ala Ile 195 200 205			624
ctc ttg gtg ctg gtc ttt atc aaa aag gtg gcc aag aag cca acc aat Leu Leu Val Leu Val Phe Ile Lys Lys Val Ala Lys Lys Pro Thr Asn 210 215 220			672
aag gcc ccc cac ccc aag cag gaa ccc cag gag atc aat ttt ccc gac Lys Ala Pro His Pro Lys Gln Glu Pro Gln Glu Ile Asn Phe Pro Asp 225 230 235 240			720
gat ctt cct ggc tcc aac act gct gct cca gtg cag gag act tta cat Asp Leu Pro Gly Ser Asn Thr Ala Ala Pro Val Gln Glu Thr Leu His 245 250 255			768
gga tgc caa ccg gtc acc cag gag gat ggc aaa gag agt cgc atc tca Gly Cys Gln Pro Val Thr Gln Glu Asp Gly Lys Glu Ser Arg Ile Ser 260 265 270			816
gtg cag gag aga cag tga Val Gln Glu Arg Gln *			834
275			

&lt;210&gt; 12

&lt;211&gt; 277

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 12

Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr 1 5 10 15
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu 20 25 30
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val 35 40 45
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu 50 55 60
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His 65 70 75 80
Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr 85 90 95
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr 100 105 110
Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly 115 120 125
Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu

130						135						140					
Pro	Cys	Pro	Val	Gly	Phe	Phe	Ser	Asn	Val	Ser	Ser	Ala	Phe	Glu	Lys		
145						150					155				160		
Cys	His	Pro	Trp	Thr	Ser	Cys	Glu	Thr	Lys	Asp	Leu	Val	Val	Gln	Gln		
						165					170				175		
Ala	Gly	Thr	Asn	Lys	Thr	Asp	Val	Val	Cys	Gly	Pro	Gln	Asp	Arg	Leu		
			180						185				190				
Arg	Ala	Leu	Val	Val	Ile	Pro	Ile	Ile	Phe	Gly	Ile	Leu	Phe	Ala	Ile		
		195					200					205					
Leu	Leu	Val	Leu	Val	Phe	Ile	Lys	Lys	Val	Ala	Lys	Lys	Pro	Thr	Asn		
	210					215					220						
Lys	Ala	Pro	His	Pro	Lys	Gln	Glu	Pro	Gln	Glu	Ile	Asn	Phe	Pro	Asp		
225					230					235					240		
Asp	Leu	Pro	Gly	Ser	Asn	Thr	Ala	Ala	Pro	Val	Gln	Glu	Thr	Leu	His		
					245				250					255			
Gly	Cys	Gln	Pro	Val	Thr	Gln	Glu	Asp	Gly	Lys	Glu	Ser	Arg	Ile	Ser		
			260					265					270				
Val	Gln	Glu	Arg	Gln													
		275															